Review: Multiple Roles of Cord Factor in the Pathogenesis of Primary, Secondary, and Cavitary Tuberculosis, Including a Revised Description of the Pathology of Secondary Disease

Robert L. Hunter, Margaret R. Olsen, Chinnaswamy Jagannath, and Jeffrey K. Actor
Department of Pathology and Laboratory Medicine, The University of Texas – Houston Medical School, Houston, Texas

Abstract. Tuberculosis, once thought to have been controlled, is now resurgent in many parts of the world. Many gaps exist in understanding the pathogenesis of tuberculosis, especially secondary and cavitary disease. Evidence presented here suggests that cord factor (trehalose 6,6'-dimycolate, TDM) is a key driver of these processes. It is the most abundant lipid released by virulent \textit{M. tuberculosis} (MTB) and can switch between two sets of activities. On organisms, TDM is non-toxic and protects them from killing by macrophages. On lipid surfaces, it becomes antigenic and highly toxic. Caseating granulomas, the hallmark of primary tuberculosis, develop from interaction of TDM with lipid within granulomas. New evidence indicates that secondary tuberculosis begins as a lipid pneumonia that accumulates mycobacterial antigens and host lipids in alveoli before developing conditions for activation of the toxicity and antigenicity of TDM. This rapidly produces caseation necrosis that leads to cavities. Finally, virulent MTB release large amounts of TDM during growth as a pellicle within cavities. We propose that such growth results in activation of the toxicity and antigenicity of TDM at the air interface and that presence of the activated TDM perpetuates the cavity.

Keywords: tuberculosis, mycobacteria, cord factor, trehalose dimycolate

Introduction

Tuberculosis, once thought to be controlled, is now resurgent in many parts of the world. \textit{M. tuberculosis} (MTB) currently infects one third of the world’s population \cite{1}. It is endemic in every country of the world and it kills more people today than any other bacterial infection. Surprising gaps remain in our knowledge of tuberculosis, especially the pathogenesis of caseating granulomas (the most characteristic lesion) and of secondary tuberculosis (the stage of disease responsible for 80\% of the disease and nearly all transmission of infection). It has long been known that MTB are able to evade host defenses for long periods before activating immune responses that cause most tissue damage of tuberculosis. However, there is little understanding of how the organism induces its host to respond differently at different times during infection. This paper presents evidence that cord factor (trehalose 6,6'-dimycolate, TDM) is key to understanding these phenomena.

TDM, the most abundant lipid produced by virulent MTB, has long been a puzzling substance because it changes from non-toxic to highly toxic when injected in an oily vehicle. Our studies began with investigations of the structures of the toxic and non-toxic forms of TDM. These were followed by studies of the mechanisms of toxicity and finally by studies of the effects of TDM on tuberculosis. During the course of this work, three observations were made that contradicted widely held beliefs and provided the basis for new models that have greatly advanced our understanding of the pathogenesis of tuberculosis.
First, we discovered that changes in conformation cause TDM to switch between two sets of biologic activities. In a micellar conformation, TDM is non-toxic and protects organisms from host defenses. In a monolayer conformation, TDM becomes highly toxic and immunogenic. Second, we discovered that mice injected with TDM are able to produce caseating granulomas. This provided the first model of these lesions that can be experimentally manipulated rather than simply observed. Third, we rediscovered that secondary tuberculosis in humans typically begins as a lipid pneumonia, rather than as a granulomatous disease. The pneumonia abruptly undergoes necrosis to produce cavities that initiate the final phase of infection. Together, these findings suggested a new synthesis of the pathogenesis of tuberculosis. This manuscript will first describe the relevant features of MTB and the diseases it produces, followed by descriptions of the structure and activities of TDM, and finally by a discussion of the effects of TDM in primary, secondary, and cavitary tuberculosis.

Life cycle of tuberculosis. MTB is an obligate human parasite. It can infect most mammals, but can be transmitted only by humans. People are more resistant to tuberculosis than most animals. Only about 2% of immunocompetent people become ill with primary tuberculosis and most (90%) never develop clinical disease [2]. The animals used to study tuberculosis (mice, guinea pigs, rabbits, and monkeys), in contrast, all develop progressive primary tuberculosis and most die, but they are seldom able to transmit infection to new hosts [3]. MTB is a human pathogen because it can survive in humans for long periods and then escape to infect new people, not because it is especially virulent for humans. While there are many variations of disease, the life cycle of MTB is to infect a person to produce a transient primary infection, remain dormant for decades, and then induce secondary tuberculosis in the lung with a cavity that produces massive numbers of MTB that are coughed up to infect a new generation of people, (Fig. 1). Cavities provide a safe haven for multiplication of vast numbers of MTB with an outlet to the environment in a host that is immune to further infection. The immune system of most immunocompetent people can control primary tuberculosis effectively. However, it has no ability to eliminate secondary tuberculosis with an established cavity. Since, to avoid extinction, MTB must escape from cavities to infect new people, it seems clear that organisms have been selected for their ability to induce secondary tuberculosis with cavities.

It has long been recognized on clinical and epidemiologic grounds that primary (first infection) and secondary tuberculosis behave as different diseases [2,4]. This has recently been confirmed with the demonstration that susceptibility to the two diseases is controlled by different genes [5]. Primary tuberculosis occurs following first exposure to infection. It typically starts in the lung and rapidly spreads to the hilar lymph nodes and then hematogenously to the rest of the body. Most cases heal spontaneously, but a few progress to disseminated disease. Secondary, or post-primary, tuberculosis, in contrast, develops in persons who have developed sufficient immunity as a result of primary infection to restrict disease to the upper lobes of the lungs. People without fully competent immune systems because of AIDS, immunosuppressive drugs, old age, genetic disorders, or other conditions...
typically develop only primary tuberculosis or a mixture of primary and secondary disease [6].

Research on primary tuberculosis is rapidly producing new insights into the disease. However, since there are no animal models of secondary tuberculosis and little human tissue becomes available for study, secondary tuberculosis has remained beyond the reach of most investigators. Consequently, textbook descriptions of the pathology and pathogenesis of secondary and cavitary tuberculosis are largely extrapolations from studies of primary tuberculosis in human tissues and animal models.

It is generally assumed that tuberculosis develops when our defenses are weakened. While there is abundant evidence that this is true for primary tuberculosis, the situation with secondary tuberculosis is not as clear. Secondary tuberculosis historically developed preferentially in immunocompetent young adults (age 15-45 yr) who had been immunized sufficiently by previous infection to have immunity that was able to protect the rest of the body from the vast numbers of organisms released from a cavity [7]. With the decline in tuberculosis in this country, first exposure is more likely to occur in the aged and in people with impaired immune systems [2,8]. This has extended the age distribution of tuberculosis towards the elderly. The primary and secondary diseases are more likely to occur together in these individuals [5]. Nevertheless, the characteristic features of primary and secondary tuberculosis remain. Susceptibility to secondary tuberculosis is a local, not a systemic, phenomenon. One lesion or even part of a lesion may progress while others regress and heal [9]. Clearly, an understanding of systemic immunity will be insufficient to explain the host response to secondary tuberculosis. The key question is how MTB first stimulates and then evades host responses to establish an infection in one part of the lung while other parts of the same lung and the rest of the body remain immune. This paper presents new evidence that TDM (ie, cord factor) is a key driver of this process.

**Biology of cord factor.** The story of cord factor began in 1947 with Gardner Middlebrook’s report that virulent MTB have a characteristic microscopic morphology known as serpentine cords [10] (Fig. 2A). Hubert Bloch proposed that formation of serpentine cords was due to a lipid on the surface of the organisms since the cords dissociated when the organisms entered oil drops [11]. He extracted viable organisms with gentle organic solvents and recovered a glycolipid from their surface that he called ‘cord factor,’ because its removal caused disruption of cords. Cord factor was later identified as trehalose-6,6’-dimycolate (TDM) [12] (Fig. 2B). Within a few years, Bloch, Noll, and their collab-

![Fig. 2. Cord factor and the formation of serpentine cords. Panel A: microscopic image of MTB showing the characteristic pattern of branching serpentine cords. This pattern of growth is associated with virulence and caused by large amounts of cord factor (TDM) on the surface of the organisms (AFB stain, 400x). Panel B: cord factor (TDM) molecules consist of one trehalose attached to two mycolic acid moieties, each of which has a short and long hydrocarbon chain. Extraction of virulent MTB by non-lethal organic solvents (eg, petroleum ether) yields nearly pure TDM [16,46].](image)

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**Table 1. Evidence that cord factor (TDM) contributes to the virulence of Mycobacterium tuberculosis (MTB).**

- TDM is present in large amounts on the surface of virulent, but not avirulent, MTB [13].
- TDM is the most abundant lipid produced by virulent MTB [11,14].
- Lipid removed by non-lethal extraction of live MTB is nearly pure TDM [15-17].
- TDM is responsible for the cording-climbing morphology of virulent MTB [13,14].
- TDM is the most granulomagenic and toxic lipid of MTB by orders of magnitude [16,18-20].
- TDM enhances both acute and chronic tuberculosis in mice, but has little effect on other infections [21].
- TDM interferes with the ability of INH to kill MTB in mice [13].
- Removal of TDM from the surface of mycobacteria reduces their ability to survive in macrophages and in mice [15,21-23].
- Loss of MTB ability to synthesize excess TDM correlates with loss of virulence [14,16,24].
- Mutations that alter the structure of mycolic acids of TDM can suppress the virulence of MTB [17].
Organized structures formed by TDM. TDM (6,6'-trehalose dimycolate) is a highly insoluble amphiphile that forms micelles in aqueous suspension or highly ordered stable configurations on hydrophobic interfaces [28,30-32]. The structure of the crystalline monolayer that forms at hydrophobe-aqueous interfaces is shown in a diagram (blue ovals represent trehalose and black lines the short and long hydrocarbon chains of mycolic acid) and by scanning tunneling microscopy at 1,840,000x magnification (top right). The major dark lines, spaced approximately 90Å, are elevated arrays of trehalose head groups. A secondary pattern intersecting the main one at an angle of 45° with a periodicity approximating 65Å represents the mycolic acid residues. Susceptible mice are killed by as little as 10 µg of TDM in this configuration. The mycobacterial cell wall is extraordinarily thick, consisting mainly of lipoarabinomannan (LAM) with mycolic acids that are oriented perpendicular to the plane of the membrane to produce the inner leaflet of an intercalated bilayer with TDM. This thick and rigid bilayer is an essential component of mycobacteria [32]. In aqueous suspension, TDM forms cylindrical micelles whose structure is shown as diagrams and scanning tunneling microscopy at 500,000x magnification (bottom right). The surface consists entirely of trehalose moieties with no exposed fatty acid moieties [28]. These micelles are non-toxic [13]. The large excess of TDM produced by virulent MTB is so insoluble that it remains on the surface of organisms—probably as micelles. It is likely that the orderly arrangement of micelles shown here is responsible for the formation of serpentine cords by virulent MTB.

Orators published evidence that cord factor contributes to the virulence of MTB (Table 1).

Even though most of the original data were promptly confirmed and have been extended by numerous investigators over the years, the idea that cord factor is a virulence factor of MTB continues to be questioned on two grounds [16,25]. First, with the development of better analytic methods, TDM was found in all mycobacteria, not just virulent MTB. The significance of differences among species in the quantity and mycolic acid structure of TDM was seldom considered. Second, the requirement of oil for expression of the toxicity of TDM was considered ‘unphysiologic’ [16]. The idea that cord factor was a virulence factor of tuberculosis was widely dismissed. This is an interesting episode in the psychology of science. Few of the data linking TDM to the virulence of tuberculosis had been challenged. The real problem was that the activation of toxicity of a glycolipid by oil could not be explained or investigated by the prevailing paradigms of science. As documented in other fields, scientists tend to dismiss experimental results that they cannot explain [26]. This situation
is typically resolved only by investigators from a different field.

Our investigations started with the observation that the amphiphilic (surface-active) properties of proteins control their ability to induce cell mediated immunity [27]. Studies on TDM were initiated to learn how surface activity of adjuvants contributed to their immunostimulatory properties. Retzinger demonstrated that TDM spontaneously formed a crystalline monolayer on hydrophobic surfaces that is more rigid and stable than that formed by any other biologic amphiphile yet described [28,29]. The monolayer formed similarly at oil-water, plastic-water, or air-water interfaces. A molecular model of the TDM monolayer was refined by Behling and Schabbing [30,31] (Fig. 3). The monolayer is a two dimensional crystal of regular linear arrays of hydrophilic (trehalose) and hydrophobic (mycolate) domains. The monolayer on beads was found to be highly stable in vitro and in vivo after injection into mice [28]. If placed in aqueous suspension without a hydrophobic interface, TDM forms cylindrical micelles [28]. These micelles have a uniformly hydrophilic surface composed of trehalose moieties. The mycolic acid groups are entirely covered. Finally, TDM is found complexed with other lipids in mycobacterial cell walls. It forms an asymmetrical intercalated bilayer with lipoarabinomannan (LAM) [32]. The size and the interlocking short and long hydrocarbon chains of mycolic acid contribute to the extraordinary stability and impermeability of mycobacterial cell walls.

The micellar and monolayer configurations of TDM have very different biologic activities (Table 2). TDM micelles are non-toxic. The largest dose of TDM ever injected into a mouse, 50,000 µg, had no adverse effect [21]. The physical state of TDM on the surface of MTB has not been adequately studied. However, electron micrographs of organisms demonstrate masses of cylindrical structures with the dimensions of micelles and no evidence of a monolayer [33]. These images together with the free energy requirements of the monolayer suggest that TDM free on the surface of MTB exists primarily as micelles [28,29]. TDM on MTB prevents phagosome/lysosome fusion, prevents acidification, and protects the organisms from killing by macrophages [15]. Removal of lipids (primarily TDM) from the surface caused 99% of the organisms to be killed within 3 days by human or mouse macrophages in culture. Adding back purified TDM almost completely restored the ability to organisms to survive in macrophages [15]. Recent data suggest that TDM on MTB also impedes antigen presentation, probably as a result of altered trafficking within phagosomes [34].

The most unique and characteristic property of TDM is that it acquires a completely different set of biologic activities when it assumes the monolayer configuration. It becomes orders of magnitude more toxic and more granulomagenic than any other mycobacterial lipid, with an LD₅₀ for mice in the order of 10-30 µg [16,35]. Its toxicity is a direct function of the surface area of the monolayer and is otherwise unrelated to dose [29]. The precise chemistry of the mycolic acid residues and the size of particles that support the monolayer are important [17]. Beads >5 µm in diameter were required to induce marked inflammatory responses [36,37]. Injections of TDM in oil (the monolayer configuration) have been reported to enhance both acute and chronic murine tuberculosis and to counter the therapeutic activity of isoniazid [13].

Table 2. Activities of the monolayer and micellar forms of TDM.

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<th>TDM Micelles</th>
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<td>Conformation of excess TDM on virulent organisms.</td>
<td>Non-toxic (LD₅₀ &gt;50,000 µg).</td>
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<tr>
<td>Protects MTB from killing by macrophages.</td>
<td>Prevents phagosome/lysosome fusion.</td>
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<tr>
<td>Prevents acidification of phagosomes.</td>
<td>Induces little or no inflammatory reaction.</td>
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<td>May impede antigen presentation.</td>
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<th>TDM Monolayer</th>
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<td>Forms spontaneously on hydrophobic (lipid-water or air-water) interfaces.</td>
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<tr>
<td>Toxic (LD₅₀ &lt;50 µg).</td>
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<td>Toxicity is a direct function of surface area.</td>
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<td>Kills macrophages in minutes.</td>
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<td>Induces active foreign body granulomas in naïve mice.</td>
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<td>Induces hypersensitivity granulomas in sensitized mice.</td>
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<td>T cell immunogen.</td>
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<td>Induces caseating granulomas in sensitized mice.</td>
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<td>Enhances acute and chronic tuberculosis in mice.</td>
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Studies were designed to investigate the mechanism of toxicity of TDM in the monolayer configuration. TDM spread as a molecular monolayer on plastic dishes was found to be highly toxic for macrophages (Fig. 4A) [19]. Cells adhered and spread maximally within minutes on a TDM monolayer. Cells on uncoated portions of the same dish showed little or no spreading at this time (Fig. 4B). When observed in real time, macrophages were killed by contact with the TDM monolayer in as little as 5 min after making initial contact the TDM monolayer. (Frame from video of unstained cells using confocal microscopy at 500x.) Panel D: at 24 hr, the cells had formed a continuous layer on uncoated plastic, while those in contact with TDM had been killed, leaving a large hole in the lawn of cells (Wright’s stain, no magnification). Panel E: cells at 24 hr on an area with excess TDM that had formed into ring-like structures demonstrating that the cells recognize and react to different conformations of TDM and that the conformations remain stably attached to the plastic surface (Wright’s stain, 40x). Panel F: detail of panel D showing live cells attached to a ring structure (micelle) while other cells spread massively and are killed on the intervening areas that are coated with a monolayer of TDM (Wright’s stain, 400x).

Fig. 4. Macrophage killing by contact with the TDM monolayer. TDM dissolved in hexane-ethanol (9:1, v:v) was spread in a circle on a plastic tissue culture dish in a quantity sufficient to produce a single molecular monolayer (0.37 µg/cm²). Murine macrophages (J774A.1) were then added to the dish to produce an even distribution of cells. Panel A: at 20 min, the cells on the TDM monolayer spread in 360° and some had already been killed (Wright’s stain, 400x). Panel B: at 20 min, cells in contact with the uncoated dish had only begun to attach (Wright’s stain, 400x). Panel C: macrophage with membrane blebs prior to rupture within 10 min after making initial contact the TDM monolayer. (Frame from video of unstained cells using confocal microscopy at 500x.) Panel D: at 24 hr, the cells had formed a continuous layer on uncoated plastic, while those in contact with TDM had been killed, leaving a large hole in the lawn of cells (Wright’s stain, no magnification). Panel E: cells at 24 hr on an area with excess TDM that had formed into ring-like structures demonstrating that the cells recognize and react to different conformations of TDM and that the conformations remain stably attached to the plastic surface (Wright’s stain, 40x). Panel F: detail of panel D showing live cells attached to a ring structure (micelle) while other cells spread massively and are killed on the intervening areas that are coated with a monolayer of TDM (Wright’s stain, 400x).

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The stability of the monolayer and micellar configurations of TDM and their effects on macrophages were demonstrated in a single preparation. If TDM in a solvent is placed on a plastic dish in concentrations larger than a monolayer, then the excess forms ring-like micellar structures within the monolayer [19]. Macrophages adhered avidly to the ring structures and survived, while those on adjacent areas of monolayer spread rapidly and were killed (Figs. 4E, 4F). This experiment demonstrates that different conformations of TDM are stable and that macrophages react differently to them. Macrophages are killed on the monolayer, but not on the micellar rings of TDM.

Injections of TDM on beads or oil emulsions into naïve mice induce granulomas reminiscent of early tuberculosis [20,38-40]. They consist of activated macrophages and are accompanied by systemic production of TNF-α. Fibrinogen is a cofactor for induction of granulomatous inflammation by TDM [29]. Injections of multiple doses produce cachexia reminiscent of chronic tuberculosis. This systemic toxicity is accompanied by
loss of ability to produce granulomas at the site of injection, dissemination of beads hematogenously throughout the body, and finally death from hemorrhagic pneumonia [41, 42].

Tissue damage in tuberculosis has long been associated with hypersensitivity directed against protein antigens, especially PPD [2,9]. A surprising finding is that TDM can also be a potent T cell immunogen [18,43]. Mice immunized by low dose infection with mycobacteria or by TDM complexed to methylated BSA produce hypersensitivity granulomas when challenged with an oil emulsion of TDM [18,34]. The ability to produce such granulomas can be passively transferred by CD3+, CD-1 restricted T cells from immunized animals. The strength of these TDM-specific responses was at least equivalent to those induced by protein antigens, suggesting that TDM may be a major T cell immunogen of MTB. The mechanisms of immunization and elicitation of responses remain unclear. Injection of TDM alone did not immunize animals to produce hyper-sensitivity granulomas [18] (unpublished). Much research will be required to understand fully the antigenic and immunogenic properties of TDM.

Role of TDM in primary tuberculosis. Since MTB is an intracellular pathogen during early stages of infection, an ability to survive and grow in macrophages is essential for its continued survival. However, MTB are readily killed by neutrophils and some macrophages [44,45]. They manage to survive in macrophages by inhibiting killing mechanisms, eg, phagosome-lysosome fusion. Indrigo reported that removal of lipids from the surface of MTB caused 99% of them to be killed in 3 days by macrophages in culture [15,46]. Adding back purified TDM restored almost completely the ability of MTB to survive in such cultures. Similar results have been reported in vivo where MTB without abundant TDM on its surface fails to survive following injection into animals [13,22, 23]. Although the details are obscure, it is clear that TDM on the surface of MTB protects the bacteria from host defenses during primary infection.

Caseating granulomas are the most characteristic lesion of primary tuberculosis. Mice have long been considered to be a flawed model of tuberculosis because they fail to produce caseating granulomas following infection with a low dose of organisms. However, we recently reported that ip injections of a high dose of MTB or an oil emulsion of TDM into sensitized mice induced typical caseating granulomas in mesenteric adipose tissue [44]. Variations in protocols resulted in a series of caseating granulomas, which reproduced morphologic and functional features of lesions of human tuberculosis. The data suggested that induction of caseating granulomas required a sufficient dose of TDM to become associated with lipid in an appropriately sensitized host. Mice infected with a low dose of MTB become appropriately sensitized, but fail to develop other conditions necessary for the production of caseating granulomas.

Since human tuberculosis is primarily a pulmonary disease, the relevance of caseating granulomas in the mesenteric fat of mice was uncertain. We recently had an opportunity for direct comparison of human peritoneal tuberculosis with the TDM induced lesions in mice (Fig. 5). The lesions were remarkably similar. Both consisted of a central core of caseous material inside concentric rings of foamy macrophages, epitheloid cells, lymphocytes, and other inflammatory cells. The lesions developed in adipose tissue that was heavily infiltrated with lymphocytes and contained Langhans-type giant cells. The caseous material of both lesions appeared to be composed largely of host lipid. This is evidence that TDM-induced caseating granulomas in mice are a valid model of human peritoneal tuberculosis.

Published reports and histologic sections of lesions of acute tuberculosis in other organs were reviewed. We found that tuberculosis characteristically develops in adipose tissue of organs that contain large amounts of such tissue. This includes the breast, skin, and bone marrow, in addition to the mesentery [47,48]. However, tuberculosis also occurs in organs that contain little adipose tissue, especially the lung. The association of lipid with active tuberculosis and caseation in these organs was examined using histologic slides of untreated human tuberculosis. Sections of tuberculous lesions in mesentery, lung, brain, liver, bone marrow, and lymph node all demonstrated that histologic evidence of active tuberculosis (Langhans-type
giant cells, acid-fast bacilli, necrosis-containing nuclear debris, and foamy macrophages) was accompanied by spherical lipid deposits (Figs. 5C, 6B). The lipid in caseous lesions in mesentery and bone marrow appeared to have been derived from adipose tissue. The lesions in liver, brain, lymph node, and lung showed comparable evidence of lipid, but were not associated with adipose tissue, implying that caseating granulomas of primary tuberculosis can utilize lipid derived from multiple sources. These findings support the hypothesis that the interaction of TDM with lipid to activate TDM toxicity is an important component of human tuberculosis. The organisms not only produce TDM, but they stimulate the accumulation of lipid suitable for activation of its toxicity. Such lipid in tuberculous lesions in extra-pulmonary sites of infection is uncharacteristic of murine tuberculosis and may explain why mice fail to produce caseating granulomas following low dose infection.

**Role of TDM in secondary tuberculosis.** In attempting to extend these studies to secondary tuberculosis, we discovered a major disconnect between descriptions of the pathology of secondary tuberculosis in contemporary and preantibiotic eras. The introduction of effective antimycobacterial drugs in the 1950’s produced a precipitous decline in the numbers of specimens available for study. Consequently, current descriptions of the pathogenesis of secondary tuberculosis are largely extrapolations from studies of primary tuberculosis in human extrapulmonary sites and animal models. Secondary tuberculosis is described as a war of attrition in which the ability of MTB to divide contends with the ability of the host to recruit and activate macrophages sufficient to kill them.

![Fig. 5. Comparison of human mesenteric tuberculosis with TDM-induced caseating granulomas in mice. Sections of human peritoneal tuberculosis (panels A, B, and C) are compared with caseating granulomas induced by ip injection of TDM in its toxic form in oil into mice (panels D, E, and F) [44]. Caseating granulomas developed in adipose tissue in both lesions (panels A and D). The caseating granulomas consisted of a central core of caseous necrosis surrounded by concentric layers of foamy macrophages, epitheloid macrophages, and finally lymphoid and other inflammatory cells. In other areas, adipose tissue contained a dense infiltration of lymphoid cells (panels B and E). Foci of early caseation were observed within the mass of lymphocyte-infiltrated fatty tissue. Finally, both lesions contained Langhans-type giant cells (panels C and F). (Panels A, B, D, and E, 40x; panel C, 400x; panel F, 600x; H&E stain.)](image-url)
Cavities are thought to arise from caseating granulomas that enlarge centrifugally because incoming macrophages cannot be activated rapidly or sufficiently to stem the growth of MTB. They die adding to the caseous core. The lesions progressively enlarge until they erode into a bronchus to form cavities. While this description is widely quoted, there is little or no direct evidence that this is an accurate description of the pathogenesis of secondary tuberculosis.

Multiple studies of the pathology of human tuberculosis from the late 19th century to the 1950’s described a process quite different from that found in modern texts [7,49-51]. They reported that secondary tuberculosis starts as a lipid pneumonia. Modern pathology texts describe tuberculous pneumonia only as a result of spillage of infected material from cavities, not as a precursor to them. The earlier investigators reported that alveolar macrophages containing abundant stainable lipid accumulated within alveoli and that lymphocytes infiltrated alveolar septa usually of a peripheral portion of an upper lobe [52]. The pneumonia produced few signs or symptoms for many months.

Fig. 6. Secondary tuberculosis with early cavitation. This person had been ill for 2 wk with fever and chest pain, but refused to see a doctor and died at home. Autopsy revealed tuberculosis in the upper lobes of both lungs with early cavitation. Panel A: section of tuberculous pneumonia demonstrating classic changes of endogenous lipid pneumonia as described in the preantibiotic era [7,50,51]. There is no change in the lung structure or damage to alveolar walls. The alveoli are filled with foamy macrophages, fibrin, and cell debris. A spectrum of changes was observed in alveoli ranging from all viable foamy macrophages to packed lipid debris with early organization (H&E, 200x). Panel B: adjacent section showing an alveolus with lipid-laden macrophages and a Langhans giant cell (H&E, 400x). Panel C: section of the same lung showing the wall of a cavity near the pleural surface. There were no granulomas in these sections. The cavity wall consists of fibrin and fibrous tissue overlying granulation tissue and alveoli filled with lipid and foamy macrophages. Necrotic, apparently infarcted, lung tissue is present within the cavity (top) (H&E, 40x). Panel D: section of the same lung showing obstruction of a bronchus with bronchocentric tuberculosis (H&E, 40x). Panel E: section of the same lung showing obstruction of a bronchus with lipid-laden macrophages and debris surrounded by bronchocentric tuberculosis (H&E, 40x). Panel F: section of nearby Langhans giant cell (H&E, 400x).
until it suddenly developed massive necrosis, sometimes of a whole lobe of lung, to produce a cavity from which the disease progressed.

While the ability of pathologists to study untreated secondary tuberculosis declined in the past half century, that of radiologists was enhanced by new technology, especially CAT scans. Today, radiologists clearly differentiate primary from secondary tuberculosis and report that secondary tuberculosis begins as a pneumonia, not as progressively expanding caseating granulomas [53-56]. Recent articles published in Eastern Europe and China confirm that secondary tuberculosis can begin as a pneumonia [57,58].

Stimulated by these reports, we initiated a search for tissues from patients with untreated tuberculosis. Lung sections from a middle-aged person who died following a brief 2-wk illness were particularly informative (Fig. 6). They demonstrated several stages of lipid pneumonia in which alveoli were filled with foamy, lipid-laden, or degenerating macrophages and frequent Langhans giant cells (Figs. 6A, 6B). The alveolar walls were mostly uninvolved. Newly formed cavities were present in other sections (Fig. 6C). They appeared to result from necrosis of tuberculous pneumonia, rather than from caseating granulomas. Necrosis was associated with vasculitis and thrombosis of small to medium vessels and appeared to result from a combination of caseation and infarction. Such lesions are characteristic of delayed-type hypersensitivity [59]. Lesser involved areas of lung demonstrated endobronchial tuberculosis (Figs. 6D, 6E, 6F).

The combination of endobronchial tuberculosis and focal central lobular tuberculous pneumonia is responsible for the most characteristic radiologic feature of active secondary tuberculosis, the tree-in-bud pattern [53-55]. Choi reported radiologic findings in 17 patients who, like the one we studied, presented with acute respiratory failure [60]. The radiologic findings in these patients were similar, but more intense, than those of patients with less severe disease and consisted of bronchogenic disseminated tuberculosis with diffuse ground glass attenuation. Ground glass attenuation with consolidation and interstitial abnormalities are characteristic of lipid pneumonia [54]. While these reports do not all include histopathology, they are entirely consistent with the sections we studied, providing additional evidence that secondary tuberculosis begins as a pneumonic process quite different from that described in most modern textbooks.

When we realized that secondary tuberculosis may begin as a pneumonia, we attempted to induce such lesions in mice [44]. Very large numbers \((10^9)\) of virulent MTB were injected directly into a lung of sensitized mice. As expected, the injections induced violent reactions. Some mice died within 3 days of hypersensitivity pneumonitis, but not from tuberculous pneumonia. The mice developed an acute inflammatory process with severe edema, not a lipid pneumonia. The animals that survived cleared organisms from alveoli completely by 7 days and appeared entirely normal at 2-3 weeks. This confirmed reports that MTB are readily killed by neutrophils [45]. It appears that MTB do not overwhelm and exhaust host defenses in secondary tuberculosis as they do in primary or disseminated tuberculosis in subjects who are unable to mount a sufficient immune response [61-63]. Rather, the MTB require an immune response sufficient to control primary tuberculosis. They somehow manipulate this response in local areas of the lung so that they can survive in foamy alveolar macrophages while they gradually induce conditions for eventual production of a cavity in order to escape to infect new hosts.

Numerous investigators have reported that tuberculous pneumonia of early secondary tuberculosis may spontaneously regress and heal completely [7,9,49,51]. Early in the course of secondary tuberculosis, it was impossible to tell which patients had disease that would progress to cavities and which would heal. Caseation necrosis and cavitation of tuberculous pneumonia occur in patients with a high degree of hypersensitivity to tuberculin and large numbers of organisms in alveoli [7,9,51]. There was no explanation of how MTB could grow to large numbers in alveoli of persons with sufficient immunity to prevent infection of other organs. It was frequently assumed that host defenses had weakened. While it is true that active tuberculosis is associated with conditions that suppress immunity, including malnutrition, diabetes, and trauma, the disease preferentially
strikes young immunocompetent adults, not the less immunocompetent aged or very young, and it affects only local areas of the lung [4,49]. Much remains to be learned.

Autopsies done in the first half of the 20th century revealed evidence of healed tuberculosis in nearly all adults [2]. Two types of lesions were found. The first was a Gohn complex of primary tuberculosis (healed calcified granulomas in the periphery of the lung and in a hilar lymph node) [7]. The second was scars in the apex of a lung known as Simon’s foci [9,64]. The identification of apical scars as healed tuberculosis has been questioned because they contain neither culturable organisms nor evidence of healed granulomas [65]. However, a high proportion contained lipids that were consistent with resolved lipid pneumonia. We interpret this as evidence that many of the scars were, in fact, due to tuberculosis. This is an important point. If the scars were all due to secondary tuberculosis, then almost all adults had the disease, but 90% regressed spontaneously before formation of a cavity. It is well known that cavities typically persist for the life of a patient and rarely heal. It is not appreciated, however, that the process that gives rise to cavities may resolve and heal spontaneously in many, perhaps most, adults. Elucidating the true incidence and mechanism of spontaneous resolution of secondary tuberculosis should be a priority area for research.

Little is known of the factors that contribute to lipid accumulation in tuberculous pneumonia, but there are models and clues. Certain mutations in lipid metabolism may be associated with increased resistance to death from tuberculosis [66]. Free mycolic acids are effective stimulators of foamy macrophages [67]. Mice with slowly progressive pulmonary or reactivation tuberculosis develop a tuberculous pneumonia that appears to be a model of early secondary tuberculosis in people [44,62,63]. MTB disappear from all parts of the body except for foamy alveolar macrophages. The infection progresses in alveolar macrophages with accumulation of host lipid and mycobacterial antigens for 1-2 yr before the lesions suddenly undergo necrosis and the animals die. A significant difference between the human and mouse diseases is that secondary tuberculosis in humans typically involves <25% of the lung and people survive, while the murine lesions involve about 80% of the lung and the animals die.

Obstruction of airways is a common pathologic and radiologic finding of secondary tuberculosis [68]. It may be an important component of secondary tuberculosis leading to the formation of cavities. Obstructive pneumonia is an endogenous lipid pneumonia usually caused by obstruction of bronchi by tumor [69]. Alveoli become filled with lipid-laden macrophages as alveolar walls are infiltrated with lymphocytes in a pattern similar to early tuberculous pneumonia. The lipid is derived from type 2 pneumocytes. In advanced stages, the lesions may liquefy to form cavities. We observed airway obstruction by endobronchial tuberculosis associated with lipid pneumonia in the case we studied (Fig. 6D). Bronchi were obstructed either with granulomas or with lipid material mixed with macrophages and acute inflammatory cells (Fig. 6E). Langhans giant cells were present (Fig 6F). Since evidence of endobronchial tuberculosis (tree-in-bud pattern) together with ground glass appearance of lipid pneumonia are the most characteristic radiologic findings of secondary tuberculosis, this is evidence that bronchial obstruction may be important in the pathogenesis of the lipid pneumonia of secondary tuberculosis [55,70,71].

**Role of TDM and lipid in caseation necrosis.** Our hypothesis is that caseation necrosis in tuberculosis is triggered by the TDM monolayer that forms on lipid surfaces [44] (Fig. 7A). A similar monolayer that forms at air interfaces may contribute to the maintenance of cavities (Fig. 7B). The requirement for oil for the expression of the toxicity of TDM was considered unphysiologic. However, there has long been evidence that association of viable MTB with oil can enhance tuberculosis in mice and probably in humans as well [72-74]. We reported that caseating granulomas in mice can be produced by interaction of TDM with lipid [44]. This paper reports that lipid is a consistent component of both active primary and secondary tuberculosis wherever it occurs. The final piece of data supporting our hypothesis is that AFB were found in lipid droplets.
associated with developing necrosis in both mouse and human tuberculosis (Figs. 7C, 7D). AFB were observed in lipid of fat cells in association with developing caseating granulomas of peritoneal tuberculosis in mice [44]. AFB were also present within lipid droplets in acutely necrotizing lesions in the lungs of mice and humans with tuberculous pneumonia. These studies demonstrate that caseation necrosis in both pulmonary and extrapulmonary sites is associated with interaction of MTB with lipid droplets in a fashion consistent with activation of the toxicity and immunogenicity of TDM. Far from being unphysiologic, the interaction of TDM with lipid surfaces is probably central to the pathogenesis of tuberculosis. Its ability to change from a nontoxic protector of organisms to the highly toxic driver of caseation necrosis is a likely contributor to the ability of MTB to direct host responses for their benefit and our detriment.

**Role of TDM in persistence of cavities.** Cavities, once formed, usually persist for the life of the host and continuously produce virulent MTB that are coughed into the environment. People who die of tuberculosis frequently have tuberculous pneumonia in addition to a cavity. However, cavities can be thin-walled fibrous structures with inflammation confined to a narrow rim near the inner surface and mycobacteria largely confined to the air interface [75,76] (Figs. 8A, 8B).

A detailed gross description of such cavities was written by Laennec [77] who discovered that they produce a characteristic sound (pectoriloquism) when examined with a stethoscope. At autopsy, the cavities were entirely empty. The walls of the cavities were covered with a semitransparent, grayish white, smooth membrane-like substance with a texture like that of cartilage, but somewhat softer, adhering closely to the pulmonary tissue. This false membrane could be thin, smooth, white, nearly opaque, of very soft consistency, and almost friable, so that it can be readily scraped off by the scalpel. The membrane was generally quite perfect with a smooth and polished surface, covering the whole internal surface of the cavity and extending into the bronchi that open into it, so that parts are apt to be detached and discharged in the sputum.

The vast majority of MTB are extracellular in fluid debris within cavities. Few hypotheses even attempt to explain this phenomena. The numbers of MTB produced in cavities can be massive. One man with “moderately severe pulmonary tuberculosis” produced between 1.4 and 4 billion bacteria/day, based on 16 measurements over 2 mo [49]. Cavities do not heal unless they are collapsed.
organisms grow almost exclusively near the interface of air with the fluid that continuously seeps into the cavity (Fig. 8C). Such organisms would be expected to grow as a surface pellicle at such an air interface. Mycobacteria were named because of their proclivity to grow as thick pellicles that resemble mold on the surface of liquid media [35] (Figs. 8D, 8E, 8F). The membrane-like lining of cavities described by Laennec [77] is consistent with a pellicle of MTB. Virulent MTB growing as or the organisms are killed by antibiotics. The factors that prevent healing are not understood.

Since cavities produce vast numbers of organisms that synthesize and release large amounts of TDM, TDM is a likely participant in their maintenance. However, compared with either caseating granulomas of primary tuberculosis or tuberculous pneumonia of developing secondary tuberculosis, cavities contain insufficient lipid for activation of the toxicity of TDM. In cavities, organisms grow almost exclusively near the interface of air with the fluid that continuously seeps into the cavity (Fig. 8C). Such organisms would be expected to grow as a surface pellicle at such an air interface. Mycobacteria were named because of their proclivity to grow as thick pellicles that resemble mold on the surface of liquid media [35] (Figs. 8D, 8E, 8F). The membrane-like lining of cavities described by Laennec [77] is consistent with a pellicle of MTB. Virulent MTB growing as
a pellicle release TDM that spreads on the surface of media as a rigid layer that forces the cords of organisms to spread across the surface and climb the walls of the flask [14] (Fig. 7B). Since the toxic monolayer of TDM forms at the air interface just as it does at an oil or plastic interface, an open cavity probably has a constantly renewing toxic and antigenic inner surface that kills macrophages, prevents healing, perpetuates the cavity, and forces organisms out of the cavity where they can be coughed into the environment. Since the monolayer of TDM is more rigid and stable than any host structure, its toxicity and antigenicity could facilitate the persistence of cavities in a host with sufficient immunity to control infection in the rest of the body. Perhaps this is the mechanism by which MTB turns people into bacterial atomizers that circulate in the community for years, spreading infection to new hosts, and insuring continued existence of the species.

Conclusions. A combination of historic and recent observations of human tuberculosis, together with research on mycobacterial glycolipids, has led to a reinterpretation of the pathology and pathogenic mechanisms of tuberculosis. This paper presents evidence that TDM-induced caseating granulomas in mice are a good model of human mesenteric primary tuberculosis; that secondary tuberculosis begins as a lipid pneumonia, not as a granulomatous disease; and that the ability of TDM to switch between sets of biologic activities is an important component of primary, secondary, and cavitary tuberculosis. This work provides new models and testable hypotheses about the pathogenesis of different stages of tuberculosis. It is hoped that further investigations will lead to a better understanding of tuberculosis and improved means to combat it.

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Trehalose dimycolate in primary, secondary, and cavitary tuberculosis


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