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GUEST COMMENTARY

Mycobacterium tuberculosis in the Extracellular Compartment: an Underestimated Adversary

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The development of lesions in pulmonary tuberculosis, the most common form of tuberculosis, is the result of the conflict between the invader, *Mycobacterium tuberculosis*, and the host. This is a standard for all infectious diseases. Tuberculosis, however, is the paradigm for diseases with multiplication of the responsible organism within macrophages and monocytes (2) and control of the infection by cell-mediated immunity (CMI) (5) orchestrated by T-cell-derived lymphokines and carried out by the effector cells, activated macrophages (31). In fact, the CMI generated in tuberculosis is so potent that on average, 90% of the immunocompetent humans infected with *M. tuberculosis* are able to contain the infection and avoid progression to clinical disease during their lifetimes (8, 33, 34). This potency of CMI may be demonstrated experimentally: guinea pigs previously infected with *M. tuberculosis* react against cutaneous reinfection with live bacilli by rapidly forming a necrotic skin lesion that subsequently resolves spontaneously (31). This reaction, now known as the "Koch phenomenon," suggests that the immune reaction mounted by infected animals is also particularly potent. In leprosy, a related disease, the responsible organism, *M. leprae*, is an obligate intracellular organism. It ensures its intracellular multiplication by inducing a specific immune paralysis, best demonstrated in the case of lepromatous leprosy, in which patients fail to develop delayed-type hypersensitivity (DTH) to the organism. In tuberculosis, while there may be immune dysregulation, specific DTH to purified protein derivative and granulomatous inflammation are prominent hallmarks of the disease. This contrast between *M. leprae* and *M. tuberculosis* begs the critical question of how *M. tuberculosis* avoids killing as a result of CMI despite its intracellular location. It is not simply an academic issue because it determines where antituberculous agents must encounter and kill *M. tuberculosis* during preventive and curative chemotherapy. To address this issue it is appropriate to review the remarkable but largely ignored work done in the past by Opie and Aronson (25), Long (15), Canetti (1), Lurie (18), and Dannenberg and Rook (7) and to recall the main steps of the pathogenesis of tuberculosis in humans, with emphasis on the events that take place after the development of acquired CMI.

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PENETRATION OF *M. TUBERCULOSIS* IN THE ALVEOLAR SPACE

Patients with a tuberculous cavity in their lungs are the principal source of tuberculosis transmission (14, 23). These patients expel aerosolized tubercle bacilli by the respiratory route and may infect any individual unfortunate enough to inhale the aerosolized bacteria. In 1934, Wells (37) postulated and demonstrated that "respiratory droplets generated by human coughs and sneezes would desiccate before impacting on surfaces, becoming particles so small they remain airborne as 'droplet nuclei,' carrying infectious human pathogens from person to person." Later it was demonstrated that the half-life of droplet nuclei was about 6 h (16). Large droplets (>5 μm) do not reach the alveolar space because they land on the ciliated epithelium of the airways and are carried up by the mucociliary escalator, swallowed, and rendered harmless (24, 30). The diameter of an infectious droplet nucleus is approximately 1 to 3 μm, and its content is one to three bacilli (29). It is unknown whether a single inhaled droplet nucleus is sufficient to cause infection in humans. It is conceivable that a single droplet nucleus could be enough, but it is well established that individuals who have prolonged exposure to smear-positive patients at close range (household contacts), and who are therefore presumed to have inhaled multiple droplet nuclei, do not always convert to tuberculin skin test positivity (4, 11, 32, 36).

INTRACELLULAR MULTIPLICATION OF *M. TUBERCULOSIS* IN ALVEOLAR MACROPHAGES

The early events following inhalation of *M. tuberculosis* involve engulfment by alveolar macrophages and unfettered intracellular growth until the onset of acquired CMI. These events have been the focus of intense in vitro and mouse studies for ~50 to 100 years (19, 27, 28, 31) and are not within the scope of the present review, which is focused on the events after the onset of acquired CMI. Despite their clinical importance, the latter have not been extensively studied, likely because they have not yet been reproduced in vitro and are difficult to reproduce in the experimental animal.

ONSET OF ACQUIRED CMI

In the alveolar space, bacilli are taken up into the phagosomes of resident alveolar macrophages. By escaping phago-

some-lysosome fusion, the intracellular bacilli are able to avoid killing and continue to multiply, eventually leading to lysis of the infected cell. The extracellular bacilli are then taken up by other macrophages and by blood monocytes that are attracted to the focus and then develop into immature macrophages. The latter cells readily ingest bacilli but are incapable of killing virulent *M. tuberculosis* or inhibiting their growth. Thus, the bacillary multiplication cycle is repeated within immature macrophages. The mycobacteria are transported to draining lymph nodes, where they multiply. The initial lesion and its inflamed lymph nodes form the so-called primary complex. It is during this initial period of intense intracellular bacillary multiplication that, in certain individuals, especially children below 5 years of age and immunosuppressed subjects, the bacilli may disperse to distant metastatic sites via lymphatics and the bloodstream.

CASEOUS NECROSIS

After about 6 weeks, the growth of tubercle bacilli rather suddenly ceases, the host becomes tuberculin positive, and caseous necrosis occurs. The caseous necrosis is the basic process of tuberculosis disease in humans. The interval from infection to tuberculin conversion is never more than 8 weeks and in general is 5 to 7 weeks (21). The onset of caseous necrosis coincides with the development of acquired immune resistance or CMI and DTH. Although CMI is the capacity of activated macrophages to kill *M. tuberculosis* and DTH is associated with tissue damage, both are the opposite sides of the same coin, as evidenced by the deficit in both components observed in human immunodeficiency virus (HIV)-positive patients with tuberculosis when their CD4 counts decline (12, 17). The paradoxical worsening of the signs and symptoms of tuberculosis in some HIV-positive patients receiving effective antiretroviral therapy is further evidence that CMI and DTH are mechanistically inseparable (12). The CD4 cell is the master piece of the immune response in tuberculosis, while the macrophage is the effector cell (2).

Nature of caseous necrosis. Caseation (caseum = cheese) is the "solid" necrosis of the exudative initial alveolar lesion and of the lung tissue surrounding the lesion. It results in alveolar destruction, but the elastic fibers of the alveolar walls and their vessels often persist within the caseous lesion. The persistence of elastic fibers is likely responsible for the hardness or the rubbery consistency of many solid caseous foci (1). A crucial phenomenon happens within the caseous lesion: the death of the majority, if not all, of the tubercle bacilli. There is a striking contrast between the high bacillary content of the lesions in which the caseation process is beginning and the limited number or lack of viable bacilli in old caseous foci (1, 25).

Mechanisms of caseous necrosis. Despite much debate over semantics, caseous necrosis is related to DTH. Activated cytolytic T lymphocytes kill *M. tuberculosis*-infected macrophages, leading to destruction of surrounding tissue. The host locally destroys its own tissue to control the uninhibited intracellular multiplication of bacilli that would otherwise be fatal (1). Although detrimental in essence, DTH or tissue-damaging activity is therefore an integral part of the host defenses. During the process, the majority of tubercle bacilli are killed, while some survive extracellularly in the solid caseous material but are unable to multiply because of anoxic conditions, reduced pH, and the presence of numerous enzymes released from the dead cells. That the caseation process is related to DTH is supported by the fact that all factors that augment DTH, for example, the mixture of bacilli with oil, also increase caseation (38). Similarly, animal species that have high DTH responses develop strong caseation and vice versa (35).

Evolution of caseous necrosis. The evolution of caseous necrosis is different from one individual to another, but the reasons for the differences are still largely unknown. The necrosis can become more or less organized or it can soften.

(i) Organization of the caseous necrosis. In a majority of cases (up to 90% of infected individuals), highly activated macrophages surround the caseous center. The bacillary antigens released by the dead bacilli expand T-cell populations. These T cells release interferon and probably other lymphokines that activate local macrophages. Such macrophages ingest and destroy the bacilli that escape from the edge of the caseum. In a resistant host, the caseous lesion is surrounded by a capsule. In time, its central part calcifies and even ossifies, especially if the caseous lesion had occurred remotely, for example, during childhood. Caseous lesions of small size can be infiltrated by sclerosis and even resorbed. Such lesions are devoid of viable tubercle bacilli (1, 25).

(ii) No organization. Some caseous lesions of a certain size can persist for long periods of time without a clearly defined capsule or modification of the caseous center (1). These lesions are intermediate between the preceding ones, which comprise healing scars, and the following ones, which are progressing. It is likely that viable extracellular bacilli persist in these caseous lesions without being metabolically active or have only limited metabolic activity. They are in a state of "latency." Evidence of the presence of live bacilli in these lesions is provided, first, by the recovery of very few CFU from such lesions during autopsy of individuals who have died from accidental causes (1, 25) and, second, by the beneficial role of isoniazid preventive therapy among infected persons who are found to have fibrotic lesions on chest radiography but who have not previously been treated with antibiotics (13).

(iii) Softening of the caseum. Lastly, in a minority of cases (up to 10% of infected individuals) (8), the hard caseum softens. The softening of the caseum is one of the most important events of tuberculosis. Because of softening, infection with *M. tuberculosis* progresses into tuberculosis, the disease (1). In some cases, the softening of the caseum is not associated with an increase in the number of tubercle bacilli, notably, when the softened caseous lesion is not open to the bronchi. However, in a majority of cases, the softening of the caseum is associated with emptying of the softened material through a communication with the bronchial tree, the formation of a lung cavity, and explosive growth of tubercle bacilli in the newly oxygen-enriched environment (1, 15). With a cough, the softened caseous material with its high bacillary content is discharged into the bronchi and subsequently to other parts of the lung and to the outside environment. Although softening of the caseum is the most serious event in the course of tuberculosis, its mechanism remains largely unknown.

EVOLUTION OF THE LUNG CAVITY

The wall of the cavity consists of an external zone of collagen, the cavity's capsule, and an internal zone of softening caseum where, because of the direct connection with the airways, the high oxygen content favors the intense multiplication of tubercle bacilli. For the first time during the course of the disease, the bacilli are free to multiply extracellularly.

Ultimately, it is the softening of the caseous tubercle and its result, the tuberculous lung cavity (15), that perpetuates the disease in humans. By coughing, the patient with a lung cavity aerosolizes and disseminates bacilli to the other parts of the lung and to the outside world.

Except in rare occasions, the tuberculous cavity does not heal spontaneously. However, a range of outcomes is possible. At one extreme the bacilli discharged from the cavity are ingested by nonactivated macrophages, in which they temporarily grow until the DTH or tissue-damaging hypersensitivity process kills the bacillus-containing macrophages and destroys nearby tissues. A new caseous focus is then created, and if the caseation process is repeated, a large part of the lungs is destroyed and the patient eventually dies. In fact, before the antibiotic era, 50% of patients with cavitary lung tuberculosis died within 2 years (8). At the other extreme, the bacilli discharged from the cavity are also ingested by macrophages. However, in a host with good CMI, immunologically specific T cells and their lymphokines activate macrophages, which are then able to kill the intracellular bacilli without excessive tissue damage. In such a host (25% of tuberculosis patients before the antibiotic era), continuous destruction of host tissue is not necessary to contain the growth of bacilli and the lesions become more or less stable (8). Intermediate between these two extreme events, another 25% of untreated patients experienced a chronic waxing-and-waning course of their cavitary tuberculosis.

IMPLICATIONS FOR CHEMOTHERAPY OF TUBERCULOSIS

The tubercle bacillus is justifiably considered an archetypal intracellular pathogen. However, the intracellular growth of *M. tuberculosis* is, in the immunocompetent human host, limited to the period preceding the development of specific CMI and DTH. As soon as *M. tuberculosis* infection becomes tuberculosis disease in humans, the CMI and DTH processes prevent substantial intracellular growth of *M. tuberculosis*. In this respect, the tuberculosis disease in humans (35) is very different from the experimental disease that follows the infection of mice and rats with *M. tuberculosis* (26), although it has similarities with that in guinea pigs (20) and rabbits (6).

From a practical point of view, it is the extracellular bacillary population and, as a top priority, those present in the lung cavity that clinicians aim to eliminate. It is this actively dividing population in the cavity, ranging well into the millions of organisms, that is most responsible for the person-to-person transmission of tuberculosis and that provides the reservoir for drug-resistant mutants. As shown in Table 1, the therapeutic armamentarium includes drugs active against organisms in different metabolic states and in different environments within the host. Used in combination, these drugs have the ability to kill drug-susceptible organisms and prevent the selection of drug-resistant mutants (10, 22). Indeed, within the first 2 months of appropriate chemotherapy, the vast majority of bacilli have been killed, virtually eliminating the risk of transmission and the selection of drug-resistant mutants. Provided that appropriate chemotherapy is continued, the major therapeutic challenge remaining is to eliminate the tiny number of viable drug-susceptible bacilli that persist despite several months of effective drug therapy. In that respect, rifampin is undoubtedly the most important drug. With the incorporation of rifampin into multidrug chemotherapy, clinicians can now achieve cure in a majority of patients within 6 months, whereas therapy with isoniazid previously required a minimum of 18 months. Although 6 months of therapy is a great benefit compared to 18 months, 6 months of therapy cannot be considered a short duration. To begin to understand why it takes months to kill a handful of persisters, we must address the issue of the nature, the metabolic status, and the location of these persisters. The special activity of rifampin against them does not close the debate regarding whether they are located intracellularly or extracellularly (9) because rifampin is as active in mice, in which tubercle bacilli are mainly intracellular, as in humans, in which they are mainly extracellular. Because of the potency of CMI and DTH in patients who have developed caseous lesions and because of the activity of isoniazid against latent *M. tuberculosis* infection (3), one is tempted to conclude that the persisters are likely to remain "latent" as intact cells with occasional spurts of metabolism (22), taking sanctuary in tiny areas of solid caseous material. However, some fraction of the bacillary population might also persist intracellularly in unusual forms in some patients (31). Whatever their condition or their location, they remain a significant adversary for the clinician and the scientist alike.

TABLE 1. Activities of the principal antituberculous agents by organism metabolic state

Drug	Activity against organisms[a]		
	Actively metabolizing	Slowly metabolizing	
		At acid pH	At neutral pH
Streptomycin	+++	0	0
Isoniazid	+++	+	0
Rifampin	++	+	+
Ethambutol	±	±	0
Pyrazinamide	0	++	0

[a] +, ++, and +++, bactericidal activities of increasing intensity; ±, bacteriostatic activity; 0, no activity.

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