Mycobacterial Disease, Immunosuppression, and Acquired Immunodeficiency Syndrome

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INTRODUCTION

The genus *Mycobacterium* includes a most successful group of intracellular bacterial parasites ranging from the obligate intracellular pathogen *Mycobacterium leprae* and the facultative intracellular parasites *M. tuberculosis*, *M. bovis*, and *M. avium* (all of which can cause progressive lung disease) to such environmental species as *M. goodii*, *M. fortuitum*, *M. terrae*, and *M. smegmatis*, which are seldom pathogenic for healthy adults (204). We now know that some of these mycobacterial species are able to cause active disease under certain circumstances (31), and between these two extremes lie a large number of opportunistic pathogens (including *M. intracellulare*, *M. scrofulaceum*, *M. simiae*, and *M. szulgai*) which can cause disseminated systemic disease if the patient has an underlying disease such as sarcoidosis, silicosis, emphysema, or Hodgkin's disease (101). Because of space constraints, no attempt will be made to discuss the extensive literature dealing with *M. leprae* infections. The reader should consult one of several excellent recent reviews for further details on this organism (17, 21).

Morphologically, biochemically, physiologically, and genetically, mycobacteria can differ so strikingly from one another that their only common feature sometimes seems to be their acid fastness (198). Collectively, they are responsible for a number of important human and animal diseases which constitute a real public health hazard in the United States, as well as in other countries worldwide (44). *M. tuberculosis* infects an estimated 5 to 8 million new cases, with 2 to 3 million deaths annually (3). Although, the incidence of pulmonary tuberculosis within the United States has declined steadily at an annual rate of about 5% over the past several decades, the organism is still responsible for 23,000 new cases annually (29). Despite the most strenuous efforts of the U.S. Public Health Service, the World Health Organization, and the Union against Tuberculosis, this disease persists worldwide (5) even in such countries as the United States, Scandinavia, United Kingdom,

Japan, and Australia, where a policy of aggressive case finding, chemotherapy, and/or community-wide *M. bovis* bacillus Calmette-Guérin (BCG) vaccination had virtually eliminated the disease from most segments of the population (44). On the other hand, this disease continues to flourish in many parts of Asia, Africa, and Central and South America despite our best efforts to contain it (38, 110). Even in the United States, tuberculosis seems to be on the increase once again, partly a result of the recent influx of refugees from Southeast Asia and the Caribbean (most of whom were tuberculin positive before entering this country) and partly due to the emerging acquired immunodeficiency syndrome (AIDS) epidemic (25, 26, 29). Thus, the once widely held belief that pulmonary tuberculosis has ceased to be an important infectious disease appears to be quite fallacious, and this disease continues to flourish in the inner cities among the homeless, the alcoholics, and the drug abusers (86). These groups are difficult to reach, to diagnose, and to treat effectively. They also have high relapse and drug resistance rates due to noncompliance with the relatively prolonged chemotherapy regimen needed to treat this disease successfully (177).

About 10% of tuberculosis patients are infected with mycobacteria other than *M. tuberculosis*, also known as atypical or nontuberculous mycobacteria, mostly *M. kansasii*, *M. avium*, *M. intracellulare*, and *M. scrofulaceum* (56, 75, 144). Taxonomists disagree as to the most suitable collective name for these organisms, but "nontuberculous" or "opportunistic" mycobacteria will be used in this review, although neither term is entirely satisfactory (198, 204). Some of these species have long been recognized as overt human pathogens (204), although many have only been recognized as such following the introduction of antituberculous chemotherapy (31, 101). They have become an increasing source of concern to the pulmonary physician due to their high resistance to most first-line antituberculous drugs (64). Surprisingly, the overall incidence of extrapulmonary disease due to these nontuberculous mycobacteria has not declined in parallel with *M. tuberculosis*; if anything,

the incidence has increased as a result of the developing AIDS epidemic (26, 144, 184).

Most of the nontuberculous mycobacteria exist as environmental saprophytes in soil and water, producing disease only following their accidental introduction into the tissues (172). However, isolates of many of these organisms have been reported from cancer and transplantation patients subjected to prolonged immunosuppressive therapy (31). With the appearance of human immunodeficiency virus (HIV) infection, it is now necessary to reappraise our earlier assessment of the pathogenic potential of many of these opportunistic mycobacteria for humans (101). This is because AIDS patients develop a number of life-threatening infections as the HIV infection progresses (9). Most of these infections are caused by facultative intracellular parasites such as *Candida albicans*, *Salmonella typhimurium*, and *Histoplasma capsulatum* and by cytomegalovirus (16). However, many of them also develop disseminated *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, *M. gordonae*, *M. xenopi*, or *M. fortuitum* infection (8, 77, 95). The source of the nontuberculous mycobacteria appears to be contaminated drinking water (74, 98, 199), and, in fact, many of these species have been isolated from the metropolitan water supplies serving some of our largest cities (68, 136). Contaminated tap water had been presumed to be the source of many of the mycobacterial isolates that appear occasionally in clinical specimens (urine, sputum, and gastric washings) taken from tuberculosis as well as apparently healthy patients (42).

Initially, the potential pathological significance of such isolates was ignored except when they were present in large numbers and in repeated specimens (203, 204). However, many of these environmental mycobacteria are capable of temporarily colonizing the nasopharyngeal and intestinal mucosal membranes of individuals drinking contaminated tap water or exposed to infectious aerosols produced from it (156, 199). Tuberculin testing surveys indicate that many apparently uninfected individuals exhibit low levels of hypersensitivity to PPD-A (purified protein derivative from *M. avium*) and PPD-B (*M. intracellulare*, formerly known as the Battey bacillus) presumably due to low-grade "infections" caused by water-borne mycobacteria (55, 70, 155). Such mycobacterial infections are not spread by direct person-to-person contact or by infected animals (138). Instead, the environment seems to be the major source of these infections within the community.

Careful examination of the early tuberculosis literature indicates occasional outbreaks of disseminated lung disease caused by opportunistic mycobacteria occurring in apparently healthy immunocompetent adults (90, 107). Some of these outbreaks took the form of miniepidemics, often limited to a single area or one hospital (2, 13, 34, 196, 200). There was no common environmental or occupational predisposing factor associated with these outbreaks, although they can be potentiated in individuals suffering from silicosis, pneumoconiosis, sarcoidosis, or emphysema, all of which can affect the level of macrophage activity expressed within the lung (42, 44, 45). The resulting infections are still relatively benign and self-limiting, often involving only a single lymph node. The distribution of these infected nodes suggests localized colonization of the nasopharyngeal and bronchial membranes, presumably by water-derived organisms (31). Infections of this sort frequently occur in infants and young children and are most frequently caused by *M. scrofulaceum* (176). However, isolates of *M. avium* and *M. intracellulare* (collectively referred to as members of the *M.*

avium complex [MAC]) may also be recovered from young children, presumably establishing themselves within the tissues before the host can achieve full immunocompetence (203, 204). Systemic disease may develop in severely malnourished or immunosuppressed patients or individuals suffering from an intercurrent viral infection (31, 75, 101). Severe MAC infections are also seen in patients with terminal kidney failure or in those undergoing kidney transplantation or suffering from terminal cancer (4, 82). Such life-threatening infections may occur even when these organisms appear to be essentially nonexistent in the rest of the community (1).

The underlying factor common to all of these mycobacterial infections seems to be prolonged exposure to some sort of immunosuppressive therapy (113, 139). Interestingly, MAC isolates from iatrogenically immunosuppressed patients generally belong to different serovars compared with those recovered from AIDS patients living within the same community. The reason for this difference is presently unclear (96, 112, 120). Such infections have also been reported in several nursing homes where geriatric infections appear to be equally distributed in men and women compared with the sharp male bias seen in *M. tuberculosis* infections in the elderly (96, 186). The reason for this difference is not at all clear (6).

Pulmonary disease in adults is usually caused by *M. tuberculosis* or *M. kansasii* (82, 154). However, infections due to *M. avium*, *M. intracellulare*, and *M. szulgai* also occur in most communities, usually with no obvious difference in distribution as we move across the country (34, 75, 95). The recent increase in the number of nontuberculous mycobacterial isolates reported in these patients could be due to a greater awareness of the existence of these organisms, along with the availability of improved methods for the isolation and identification of these relatively fastidious slow-growing organisms (32, 122). Once established within the tissues, these opportunistic pathogens can reach enormous numbers (up to 10^{10} acid-fast bacilli per g), infecting virtually every organ throughout the body. Histologically, the resulting lesions resemble those seen in miliary tuberculosis, lepromatous leprosy, or Whipple's disease (21, 173, 189). Thus, within a mere decade, our perception of many of these opportunistic pathogens has changed from that of a relatively rare, even exotic group of environmental mycobacteria to major pathogens of the immunologically depleted patient (42, 45).

The present review discusses some of the factors underlying the sudden emergence of *M. avium* and *M. intracellulare* as major pulmonary pathogens for the immunosuppressed patient (77, 125, 126). The role of HIV in this interaction has been extensively discussed in several recent reviews and will not be considered further (59, 80, 207). Rather, the discussion will center on the interactions that occur between the opportunistic mycobacteria and the host defenses during the evolution of the terminal phase of this important new immunosuppressive human disease.

EPIDEMIOLOGY OF TUBERCULOSIS AND OTHER MYCOBACTERIAL DISEASES

The costs of identification, diagnosis, and treatment of tuberculosis patients worldwide, together with the associated loss in productivity, have been estimated at more than half a billion dollars a year, an expense likely to continue well into the next century (3, 27). The monumental proportions of the problem are seen in the third world, especially

when placed in the context of malnutrition, overcrowding, substandard housing, civil unrest, limited financial resources, and medical manpower, which are characteristic of many of these countries and which continue to hamper all attempts at effective control. The major tools of tuberculosis control have not changed appreciably during the past 40 years, and radical innovative changes in both policy and technique are urgently needed if we are to bring this disease under control (3, 102, 110). At present, the most practical control measure for these third world countries remains the BCG vaccination of all infants and school children, especially in communities having persistently high tuberculin conversion rates (5). This recommendation is made despite the poor levels of protection achieved in the recent BCG trials in India (194). However, BCG vaccination alone will not eradicate this disease, and many of these countries do not have the financial or medical resources to deploy the case-finding and chemotherapy programs also essential for community-wide elimination of the disease (109). As a result, there have been demands for an accelerated implementation of recent advances in molecular biology and recombinant technology aimed at achieving better and earlier diagnosis of active (infectious) cases and the development of a more protective immune response (102). While many of the proposed approaches have great potential, it is doubtful whether many of the developing countries have the necessary financial and medical resources to use them effectively. Ultimately, solving these problems will be crucial to any effective tuberculosis control program in the United States, since any effective strategy must be applied on a worldwide basis if this disease is to be finally eliminated from this country. We must develop radically new, cheap, and effective diagnostic, therapeutic, and prophylactic tools to combat this tenacious pathogen, preferably during the pre-clinical (noninfectious) phase of the infection. Already a number of sensitive radioimmune, enzyme-linked immunosorbent, and deoxyribonucleic acid (DNA) relatedness assays (33, 62) have been developed for faster detection and identification of these mycobacteria, but we still need better mycobactericidal drugs that can be used in more effective short-term treatment regimens for these patients (3). Only by developing a multidisciplinary approach to the problem can we hope to bring this disease under control in a way which was predicted with such confidence only a few years ago (5, 27).

Pulmonary tuberculosis has changed over the past half-century from a disease primarily seen in infants and young adults to one now primarily associated with old men, alcoholics, and drug addicts (6, 86, 186). Much of the age-related shift probably relates to changes in standards of living and housing, to the pasteurization of milk products, to the earlier diagnosis and treatment of index cases, and to the widespread use of preventive chemotherapy for close contacts and tuberculin converters (38). Geriatric tuberculosis usually represents reactivation of latent disease acquired many years before. However, the reason(s) for the recurrence of this disease after so many years of latency is still poorly understood, although it presumably relates to the decline in the normal T-cell defenses known to occur during senescence (147). This decline releases the residual infection from its cell-mediated constraints and the patient develops active disease once again (6). Although the risk of reactivation occurring in any one year is very small, it is also cumulative, so that in an aging community the probability of apparently healthy individuals developing tuberculosis is likely to increase steadily with time (186, 191). Several other segments

of the community (in particular, people living in emergency shelters, on the streets, alcoholics, and drug addicts) seem to be at an increased risk of developing pulmonary tuberculosis (86). These subcultures may be particularly important because they represent an important reservoir of disease in groups notoriously difficult to reach and treat effectively (128, 177).

Extrapulmonary disease (mostly due to *M. kansasii*, *M. avium*, *M. simiae*, *M. szulgai*, and *M. chelonae*) continues to occur in a proportion of patients with a long history of lung disease, usually involving *M. tuberculosis* (75, 96). Following successful chemotherapy of the primary lung infection, the more drug-resistant mycobacterial species continue to multiply until the infection reaches life-threatening proportions (204). Less virulent species may establish themselves within a damaged lung (usually in an old pulmonary cavity), where they proliferate in an environment in which the host defenses and antibiotics are unable to reach them at therapeutically effective levels (10, 27, 144). In a few cases, a primary lung infection may develop, although the reason for this uncharacteristically aggressive behavior by these organisms remains unclear (56, 95). Disseminated infections due to nontuberculous mycobacteria are becoming an increasing public health concern with the expansion of the AIDS epidemic across the United States (10, 24–26, 30).

VIRULENCE OF MYCOBACTERIA FOR HUMANS AND EXPERIMENTAL ANIMALS

Humans are highly susceptible to tuberculosis infection, although most individuals who undergo tuberculin conversion fail to develop active disease (38, 44). The minimum size of the human infectious dose is still a matter for some debate, but studies of cross-infections seen in tuberculosis wards where the air quality was being continuously monitored suggest that infection can occur after a person has inhaled only one or two viable tubercle bacilli (145). However, the risk of developing active lung disease after a single exposure seems to be quite low, with only 3 to 5% of tuberculin converters developing active pulmonary disease (191). Under conditions of stress or in a confined environment (shipboard, prison, or nursing home), a higher incidence of active disease may occur, presumably as a result of repeated exposures to the infectious agent and the potentiating effect of various stress factors (186). In confined environments, infection of staff members becomes an important occupational hazard, and regular tuberculin testing should therefore be carried out.

When infected, most immunocompetent adults develop a small primary lung tubercle which does not progress to clinically significant proportions. The natural resistance mounted against this primary infection will depend on a number of unrelated environmental, nutritional, social, and genetic factors, any one of which can affect the immunological status of the host at the time of challenge (83). In humans, the primary lung tubercle will drain to the hilar and tracheobronchial lymph nodes, which appear to be highly efficient filters. This secondary infection triggers a local cell-mediated immune response which usually blocks the further spread of the infection to the bloodstream (44, 46). However, in a small proportion of patients, the infection will continue, reaching the liver, spleen, kidneys, meninges, joints, and bone marrow. As this systemic disease develops, secondary hematogenous spread may also occur, involving previously uninfected lobes of the lung which eventually develop open cavitory lesions, leading to hemorrhage and death (44).

Acquired antituberculous resistance is the paradigm of a purely cell-mediated immune response to an intracellular pathogen (44). Experimentally, such immunity can be transferred to syngeneic recipients by means of an intravenous infusion of splenic T cells from convalescent donors but not by hyperimmune serum harvested from the same animals. Active immunity is best induced by a live attenuated vaccine (40), although some protection can be induced by using killed bacilli suspended in Freund adjuvant. However, the latter response is always quantitatively inferior to that seen in BCG-vaccinated controls or the convalescent host (49).

The level of protection varies depending on the amount and persistence of the vaccinating infection within the lymphoreticular organs of the vaccinated host. The dominant T-cell type present in the infected spleen also varies as the immunization infection progresses (146). Different T-cell subsets can be distinguished on the basis of their cell surface membrane markers (e.g., Thy-1.2, L3T4, Lyt 2, IA^{a,b}) as well as by their sensitivity to radiation and cyclophosphamide treatment. However, not all antituberculous resistance is T-cell dependent (especially in the early phase of the infection), and some microbial growth limitation may be due to a population of nonspecifically activated macrophages which appear within the heavily infected spleen during the first weeks of the disease (107, 153). However, the activity of these cells is, at best, bacteriostatic in nature, providing the host with time to mount the more effective T-cell-mediated response before the infection reaches unmanageable proportions (47). The T-expressor cell population responsible for inducing the antibacterial immune response will be down-regulated once the logarithmic growth phase of the infecting population has been limited. This reduction limits the immunological damage which the T-cell-activated macrophages might otherwise induce within the surrounding tissues. In fact, much of the pathology associated with pulmonary tuberculosis (lung consolidation, fibrosis, and cavitation) has been ascribed to the deleterious action of immunologically activated macrophages within the chronically infected lungs. These highly efficient cells need to be limited as soon as possible (63).

As the number of T-expressor cells declines, they will be replaced by a population of memory immune T cells which both protect the convalescent host against reinfection by the homologous organism and provide some cross-protection against other related mycobacteria (146, 151). Production of these long-lived memory T cells probably depends on the presence of a residual mycobacterial population within the caseated tubercles left in the tracheobronchial lymph nodes or the spleen. At present, we know very little about these memory cells or the role they play in protecting the host against reinfection. However, they comprise a very important part of the immune system and are likely to be the subject of intensive investigation in the future.

Throughout this discussion, emphasis has been placed on the pivotal role played by immunocompetent T cells during the expression of antituberculous immunity (44). However, it should always be remembered that it is the activated macrophage which actually defends the host against this infection. In fact, the mononuclear phagocyte plays a complex multicellular role throughout the entire infection period, beginning with the uptake of the tubercle bacillus as it enters the alveolar sac. The resident alveolar macrophage is a scavenger cell which lacks the ability to kill virulent tubercle bacilli, which quickly begin to multiply intracellularly to produce a primary lung tubercle. Bacterial inactivation depends on the entry of large numbers of blood-derived mono-

cytes into the lesion. These cells are activated by lymphokines released by the specifically sensitized T cells when they are exposed to the specific mycobacterial antigen(s) within the granuloma. The resulting immunologically activated phagocytes are responsible for eventually bringing the infection under control. This T-cell response is triggered within the draining tracheobronchial lymph node when infected macrophages present sensitizing antigen(s) to those immunocompetent T cells which possess the necessary recognition markers (65). Later, these sensitized T cells migrate back into the tubercle, where they initiate the protective cellular immune response.

Macrophages also play an important immunoregulatory role by releasing a number of monokines (interleukin-1, gamma interferon, and tumor necrosis factor) which stimulate or regulate the activity of other components of the immune system (47, 87). The presence of too many activated macrophages during the early stages of the infection may delay or even suppress the T-cell response, thereby blocking the host response and prolonging the infection (107, 108). This type of interaction may be responsible for the persistent antigenic unresponsiveness (tolerance) characteristic of many mycobacterial infections (21). Many of these cellular interactions can be demonstrated experimentally, but evidence that they also play an active immunoregulatory role in the naturally infected host is still largely circumstantial (44, 87). Other factors may also be involved in this process. For instance, heavy loads of mycobacteria are known to affect normal T-cell recirculation patterns within the tissues, adversely affecting the expression of both delayed hypersensitivity and acquired resistance (27). Many of these aberrant immunological parameters are reversed as the infectious load is reduced by appropriate chemotherapy, suggesting that these irregularities may be the result rather than the cause of the chronic infection (42). Regardless of the role played by these interactions in the experimentally infected host, they assume more than mere academic interest when considered in the context of the MAC-infected AIDS patient (159). While HIV is undoubtedly the primary cause of AIDS (28, 80, 207), it seems equally unescapable that members of the MAC (together with a number of other opportunistic pathogens) contribute to the terminal state of immunosuppression seen in many of these patients (42, 45). The role of these opportunistic pathogens in the evolution of the terminal phase of this important new immunosuppressive disease is discussed further in a later section.

BCG vaccination protects the host by blocking the secondary hematogenous spread of the pathogen, thus limiting the primary infection to subclinical proportions (85). However, vaccination cannot prevent the establishment of the primary tuberculous lesion within the lung, nor does it have any therapeutic value against an already established disease (40). It does, however, reduce the likelihood of the patient developing the more severe miliary or meningeal form of tuberculosis, especially young children. For this reason, vaccination of all infants living in areas with high tuberculin conversion rates is to be recommended (194).

In any given population, a few unfortunate individuals seem unable to mount any sort of immunological response against *M. tuberculosis* (44). The resulting infection spreads quickly throughout the body, and the patient dies rapidly with a disseminated miliary type of disease. In most cases there is virtually no sign of any cell-mediated response to the pathogen on the part of the host defenses. We still know very little about the underlying reasons for this immunological aberration (44), although some such individuals can be

identified by their inability to react immunologically to repeated doses of BCG vaccine, suggesting that they fail to recognize key protective antigens associated with the tubercle bacillus. As a result, the virulent *M. tuberculosis* infection does not trigger the recall of the normally protective cellular immune response in the BCG-vaccinated host. Such individuals are not completely unresponsive to the mycobacterial antigens, however, since they often develop strong humoral responses (unfortunately of little protective value for the host), and this may even have some deleterious effect as a result of extensive *in vivo* antigen-antibody complex formation in these patients (21).

Even under ideal circumstances, the activated cellular defenses of the host seldom completely eliminate the tuberculous challenge from the tissues. In most cases, a residual nidus of infection persists within the lung virtually indefinitely. This carrier state constitutes a two-edged sword for the host: helping to maintain a population of memory immune T cells which can prevent exogenous infection by the same organism (148), while constituting a possible source of reactivational disease (endogenous infection) later in life. The latter attacks usually follow prolonged exposure to radiation, corticosteroids, or other immunotherapeutic drugs (135, 192).

M. tuberculosis is a natural pathogen of humans and monkeys. Experimentally, it can induce systemic disease when inoculated into rabbits, guinea pigs, rats, and mice, although there is no evidence that these animals are naturally susceptible to tuberculosis (39). Nonhuman primates seem to be especially susceptible to tuberculous infections, usually developing a rapidly fatal systemic disease (94). Experimental virulence assays with *M. tuberculosis* have been carried out in several animal species (40, 94). Most early tuberculosis studies were carried out in rabbits and guinea pigs (118, 129), but more recently a number of inbred mouse strains of varying susceptibility to tuberculosis have been used (149). Two innately susceptible (*Bcg*^s) strains of mouse, BALB/c and C57BL/6, develop progressive systemic disease after the introduction of a few hundred virulent *M. tuberculosis* into the lungs (37, 148). This level of susceptibility is much less than that reported for either rhesus monkeys or Hartley guinea pigs, both of which can be killed by an inoculum of <5 virulent tubercle bacilli (94, 105). Initial studies carried out in outbred Hartley guinea pigs had indicated that they could be killed by a single viable unit of *M. tuberculosis* H₃₇Rv (158). However, this level of virulence is difficult to maintain in laboratory cultures, and later studies indicate that the median lethal dose for this strain is now around 5×10^5 (52, 187). Even higher inoculum doses are needed ($>10^7$ colony-forming units [CFU]) if inbred strain 2 or 13 guinea pigs are used, although the reason(s) for this increase is still not clear (V. Montalbino and F. M. Collins, unpublished data).

VIRULENCE ANTIGENS OF TUBERCLE BACILLI

Despite a great deal of study, we know very little about the virulence antigens associated with *M. tuberculosis* or the way in which they antagonize the normal host defenses (63). Virulent tubercle bacilli produce a number of toxic factors (Wax D, cord factor, and sulfatides) which may damage heavily infected tissues. When introduced as purified products in large quantities into experimental animals, they are highly toxic (97). In contrast, even heavy suspensions of heat-killed tubercle bacilli (whether grown *in vivo* or *in vitro*) induce few cytotoxic effects when injected into normal mice

FIG. 1. Growth of *M. tuberculosis* Erdman (■), Indian (●), and H₃₇Ra (◆) and *M. bovis* BCG (▲) in the lungs of C57BL/6 mice following an aerogenic challenge. Vertical bars represent $\pm$ standard error for five determinations.

(39, 51, 197). Virulent *M. tuberculosis* produce several so-called virulence antigens which appear to be absent or limited in the corresponding avirulent species, especially when the organisms are grown *in vivo* (180). Thus, mycobacteria harvested from mouse lung are said to be substantially more virulent for experimental animals than the corresponding *in vitro* grown cells (50). However, this difference may be due to excessive clumping by mycobacteria harvested by differential centrifugation of spleen or lung homogenates rather than to their excessive content of "virulence" antigens.

Studies in mice have shown that nontuberculous mycobacteria are surprisingly nontoxic for the normal host even when the organisms have reached counts as high as 10^{10} CFU per g of tissue. In many cases there will be no obvious sign of disease, perhaps because the bacterial infection induces a relatively minor mononuclear cell response. As a result, the host tends to survive for long periods of time (153). When death does occur, it seems to be due to progressive consolidation and fibrosis of the lung rather than to any direct toxicity on the part of the mycobacteria towards the host tissues (141).

The relative virulence of different mycobacterial species can best be quantified in terms of the rate of growth by the organisms within the lungs, both before and after any immune response has developed within the liver and spleen (40). This parameter can be studied best in aerogenically challenged C57BL/6 mice or Hartley guinea pigs (41, 105). The higher the virulence of a test strain, the longer the logarithmic growth phase within the lung will be before it passes into a prolonged plateau phase leading to the establishment of a persistent carrier or latent infection state. The growth rate for less virulent strains may initially be similar to that for the virulent pathogen, but it will slow as the infection develops (Fig. 1) in mice infected with the Indian strain of *M. tuberculosis* compared with those infected with highly virulent *M. tuberculosis* Erdman. At first, both organisms grow

at almost identical rates, but as the infection progresses the growth curve for the Indian strain slows perceptibly, while that for the Erdman strain continues to increase logarithmically until the animal succumbs. This apparently minor difference in the late growth rate is sufficient to account for the substantial differences seen in survival times for the mice and can be taken as a measure of the virulence of these two strains. *M. tuberculosis* H₃₇Rv grows in vivo at a significantly ($P < 0.01$) faster rate than (attenuated) BCG or the avirulent *M. tuberculosis* H₃₇Ra. In fact, H₃₇Ra is unable to persist in vivo (Fig. 1) and induces little or no immunity against a subsequent virulent challenge infection (37). On the other hand, the attenuated BCG strain does multiply extensively within the spleen and induces a substantial level of antituberculous immunity as a result.

Thus, the major difference between the virulent and avirulent strains of tubercle bacillus lies in the ability of the former to multiply within the spleen long enough to induce a detectable protective T-cell response. Because of this, virulent tubercle bacilli may induce a quantitatively superior response to that achieved by the attenuated BCG, presumably because of the larger amount of antigen (immunogen or sensitin) introduced into the tissues by the former while persisting in vivo for a longer period of time (40). This phenomenon appears to be true also for different substrains of BCG which vary in their protective qualities in proportion to the amount and persistence of growth seen within the spleen (123). However, the resulting immune T cells which can be harvested at the peak of the response to fully virulent *M. tuberculosis* or the attenuated BCG can adoptively protect naive recipients against a subsequent virulent challenge (148, 151). This protective effect can be ablated by treatment of the host with isoniazid beginning at the time of vaccination, indicating that the vaccinating organisms have to be in a metabolically active state to induce an effective immune response (44).

Even under ideal circumstances, BCG vaccination does not protect all individuals subjected years later to a tuberculosis challenge. However, the severity of the infection will usually be moderated substantially, so that a smaller portion of the BCG-vaccinated individuals develop the meningeal or miliary form of this disease. They will also develop a persistent latent disease, and individuals living in countries with high endemic rates of tuberculosis are likely to possess sufficient antituberculous resistance to block intestinal colonization by MAC members (42). This cross-protective effect may help to explain why few Haitian and African AIDS patients develop systemic MAC infections while frequently suffering from *M. tuberculosis* complications (163).

VIRULENCE ANTIGENS OF NONTUBERCULOUS MYCOBACTERIA

At present, we know very little about the virulence antigens associated with nontuberculous mycobacteria (45). Such antigens are presumably responsible for the persistence of the more virulent members of this group within the lymphoreticular organs of the host (51). Although *M. kansasii*, *M. avium*, and *M. ulcerans* are able to induce progressive disease in normal immunocompetent adults, they are generally considered to be less pathogenic for humans than *M. tuberculosis* (76, 113). In general, nontuberculous mycobacteria tend to infect young children and immunodepleted adults who have ingested contaminated food or water (74, 199). A number of mycobacterial species (*M. intracellulare*, *M. scrofulaceum*, *M. gordonae*, and *M. fortuitum*) have

been recovered from domestic tap water supplies (67), where they may survive for long periods of time due to their remarkable resistance to chemical disinfectants (43, 68). Hospital drinking water supplies may be a frequent source of intestinal colonization in HIV-infected individuals, while aerosols generated during showering with contaminated tap water may directly infect their lungs. The overall importance of the oral infection route for these organisms can be inferred from the cervical lymphadenitis which develops in young children (117, 176), as well as the occasional presence of environmental mycobacteria in bronchial lavage fluids, gastric washings, and sputum samples collected from tuberculosis patients and apparently healthy adults (171, 195, 196).

On first isolation from the tissues, *M. avium* produces thin translucent colonies (virulent) when cultured on Lowenstein-Jensen egg medium or Middlebrook 7H10 agar (178). However, as these strains are cultivated on laboratory media, colonies with a domed and opaque appearance tend to appear (attenuated), and eventually rough (avirulent) variants may also develop. This type of colonial variation has been correlated with a progressive loss of mouse virulence (45, 179) as well as with differences in susceptibility to antituberculous drugs and mycobacteriophages and to the plasmid content of these organisms (57, 139, 142). Environmental isolates of *M. intracellulare* may lack plasmids, generally form domed colonies, and are avirulent for mice (89). In contrast, isolates from AIDS patients tend to form thin translucent colonies and usually bear a number of plasmids (58). Although these observations appear to exclude the environment as the source of MAC infections in AIDS patients, these differences may be reversed by selective pressures exerted by the host defenses on MAC strains which reach the gut-associated lymphoid tissue (GALT) organs of pre-AIDS patients. Mucosal macrophages may select any translucent variants which happen to be present, and these more virulent organisms will then multiply within the immunodepleted host. This effect can be demonstrated experimentally in mice infected with a mixture of translucent and opaque colony variants of *M. intracellulare* which yield almost pure cultures of the translucent colony form as the infection develops and the host defenses eliminate the opaque variant (45). Presumably, a similar type of selection process occurs within the AIDS-related complex (ARC) patient who has ingested environmental mycobacteria, a small proportion of which are translucent colony types.

IMMUNOSUPPRESSION AND ACQUIRED ANTITUBERCULOUS RESISTANCE

Vaccination of human subjects against pulmonary tuberculosis is usually carried out by the intradermal inoculation of a standardized suspension of live *M. bovis* BCG. Ideally, this is administered to infants or young school children before they have been exposed to a virulent tuberculosis infection (194). The vaccinating organism must multiply extensively at the site of inoculation as well as in the draining lymph node(s). Some viable bacilli may even reach the spleen, where they will give rise to a self-limiting systemic infection (46). The resulting protective immune response is usually monitored as a tuberculin skin hypersensitivity which develops several weeks after vaccination (44). Experimentally, this can be quantitated as an antibacterial immune response which develops within the spleen and is capable of limiting the further growth of the vaccine (or a challenge inoculum) in vivo (40). Resistance is quantitated as a substantial downward shift in the slope of the mycobacterial

growth curve in both the spleen and the lung. If splenic T cells are harvested from the vaccinated host at this time, they will protect T-cell-depleted syngeneic recipients against a fully virulent *M. tuberculosis* challenge (148). This protection can be completely ablated by pretreatment of the cells with monoclonal anti-Thy-1.2 antibody plus complement. In the absence of these immune T cells (as in athymic nude mice or anti-Thy-1.2-treated animals), a rapidly fatal tuberculous pneumonia develops even when an attenuated strain such as BCG is used as the challenge organism (141).

We still know surprisingly little about the nature of the immune response which develops against infection by the nontuberculous mycobacteria (45). Some species induce a chronic lung infection in normal mice which may last for the lifetime of the host (51). More virulent *M. avium* species may induce a life-threatening disseminated disease (34, 39, 178), while most strains of *M. intracellulare* are less virulent and tend to induce chronic pulmonary infections in Bcs^s C57BL/6 mice and its beige mutant (88, 153). These infections are substantially exacerbated by an absence of T cells (as in congenitally athymic *nu/nu* mice), the disease changing from a relatively indolent lung infection to a rapidly fatal (miliary) type of disseminated disease similar to that seen with *M. tuberculosis* Erdman (53). T-cell depletion has less effect on the in vivo behavior of mouse avirulent species such as *M. scrofulaceum*, *M. vaccae*, or *M. fortuitum* (42, 45), presumably because these species are susceptible to the bactericidal action of unstimulated mouse macrophages. Thus, the growth behavior of the avirulent mycobacteria in vivo is not substantially affected by T-cell depletion (R. W. Stokes and F. M. Collins, unpublished data). Based on such experimental findings, one would predict that avirulent mycobacteria would not be recovered from the AIDS patient population. However, some environmental mycobacterial species (*M. gordonae*, *M. xenopi*, and *M. fortuitum*, for instance) have been reported in AIDS patients (20, 56, 71), although they do not appear to be responsible for life-threatening disseminated disease as is the case for *M. tuberculosis* or MAC serovar 4 and 8 infections (118). This finding suggests that factors other than simple T-cell depletion may be important during the establishment of mycobacterial infections in the HIV-infected patient (125).

Virulent *M. avium* and *M. intracellulare* serovars may persist indefinitely within the GALT of the ARC patient. These organisms may induce a state of tolerance to specific mycobacterial antigens which, in turn, contributes to the mounting immunosuppression within the HIV-infected tissues. Prospective studies in which the intestinal contents of a large number of high-risk individuals are routinely cultured for mycobacteria could provide the necessary evidence of the existence of local gut infections in the HIV-infected individual long before the tissues are infected sufficiently to render the host tolerant to the MAC sensitins. Since many of these individuals are likely to be poorly responsive to standard purified protein derivative (PPD-S), skin tests should be routinely carried out with both PPD-A and PPD-B to provide more definitive data (55). Epidemiological studies of the distribution of important MAC serovars in the natural environment and water supplies should be correlated with isolation data for feces and bronchial lavage fluid collected from high-risk individuals before and after they become HIV positive. This type of painstaking isolation data could place us in a much better position to predict which ARC patients are likely to develop life-threatening MAC infections later in their disease. Plasmid fingerprinting and phage typing of the isolates could also help in identifying those opportunistic

mycobacteria most likely to be responsible for triggering the development of clinical AIDS in the HIV-positive individual (58, 89).

TUBERCULOSIS AND EFFECT OF IMMUNOSUPPRESSION

Pulmonary tuberculosis ranges from a self-limiting subclinical infection involving a single lung lobe (and perhaps its draining lymph node) to a rapidly disseminating, systemic disease involving virtually every organ throughout the body (40). The host-parasite interactions responsible for these two polar forms of disease are still poorly understood despite a great deal of study. Miliary tuberculosis has been associated with immunological immaturity or immunodeficiency (infants born to tuberculous mothers or arthritics subjected to intensive corticosteroid therapy). However, a number of other nonspecific factors, such as malnutrition, silicosis, sarcoidosis, hemophilia, diabetes, and terminal kidney disease, also contribute to this state (4, 12). In addition, patients suffering from leukemia or lymphoma are frequently subject to severe tuberculous complications, presumably exacerbated by the whole-body irradiation and cancer chemotherapy used to treat them (82, 140, 154). Such patients suffer from a persistent loss of skin hypersensitivity (tuberculin anergy), presumably as a result of the heavy antigenic load within the liver, spleen, and lymph nodes (21). The lesions in the anergic host consist of innumerable small granulomas containing loose aggregations of large foamy macrophages, often packed with acid-fast bacilli. There is usually little or no lymphocytic response. Such individuals are not completely unresponsive to the mycobacterial infection, however, and many patients develop a severe hypergammaglobulinemia with antigen-antibody complex formation (202).

Although there is little indication that susceptibility to tuberculosis is a genetically determined human trait (83), genetically determined differences have been demonstrated in both rabbits and mice (130, 149). The so-called *Bcg* gene effect can best be seen early in the infection before an appreciable T-cell response has developed and depends on the size and the route of the infection, as well as on the bacterial strain being tested (152). The C57BL/6 mouse is innately susceptible to an *M. avium* challenge, while the *Bcg^r* A/J or DBA/2 strain of mice tends to restrict the growth of this mycobacterium, especially in the spleen (53, 152). This can also be shown for *M. intracellulare* given by the intravenous, subcutaneous, aerogenic, or intragastric route (Fig. 2). Introduction of large numbers of viable mycobacteria (10^8 to 10^9 CFU) by the oral route will result in only minimal pulmonary involvement so that the mice survive virtually indefinitely. On the other hand, introduction of small numbers of viable bacilli (10^3 CFU) directly into the lung will result in death of most of these mice within 6 months. When the challenge inoculum was increased 100-fold by the intravenous route, there was relatively less pulmonary involvement and the mean survival time was increased to approximately 9 months. Analogous infection studies carried out in the innately resistant A/J strain of mouse produced substantially similar growth data, although the animals showed substantially prolonged survival times due to the slower growth by the challenge inoculum within the lungs of this mouse strain (53, 152).

VIRULENCE ANTIGENS AND PROTECTIVE VACCINES

We know surprisingly little about the specific immunizing antigens (or their epitopes) responsible for triggering the

FIG. 2. Growth of *M. intracellulare* in C57BL/6 mice following infection by intravenous (10^6 CFU), subcutaneous (10^6 CFU), aerogenic (2×10^4 CFU), and intragastric (3×10^8 CFU) routes. Symbols: spleen (■); lungs (▲); footpad (◆); mesenteric lymph node (◇).

antituberculous immune response by the infected individual. Several protein antigens have been isolated from both virulent and attenuated mycobacteria which produce a tuberculinlike skin reaction in BCG-vaccinated guinea pigs and mice (48, 209, 213). However, the function of these proteins within the actively growing bacterial cell is still unclear. One 65-kilodalton protein, sensitin, shows considerable homology with heat shock (stress) proteins produced by many different types of cell in response to a variety of environmental insults (127). The highly conserved nature of the genes responsible for these proteins suggests some sort of basic cellular function which is vital to the survival of the stressed cell (211). However, it is not clear at present whether the group of so-called protective proteins (71, 65, and 38 kilodaltons) released by the stressed organism can all stimulate the same cellular immune response, nor is it known why these proteins induce a cellular rather than a humoral immune response in the infected host (210). The relationship among the humoral, allergenic, and immunogenic properties of these proteins has yet to be established, and their role in the development of a protective immunity has yet to be determined (182, 209).

Perhaps the most important epitope on the protective antigen is the one associated with the activation of the memory T-cell population. These epitopes may be present in both homologous (*M. tuberculosis*) and heterologous (*M. avium* and *M. kansasii*) antigens since cross-protection studies suggest that these species are all effective immunogens in suitably infected animals (151, 153). However, there is no a priori reason to equate virulence with "protective" antigens in these organisms since the attenuated BCG vaccine seems just as protective as highly virulent *M. tuberculosis* when tested in adoptive protection studies (40, 146, 150). Thus, immunization with DNA recombinants which produce the so-called virulence genes of *M. tuberculosis* (182, 213) may induce no more protection than live BCG vaccine. Furthermore, most of these recombinant vaccines

have been prepared with antigens selected with monoclonal antibodies developed against killed or sonicated mycobacterial cells. Genes (or genomic libraries) responsible for the production of these antigens can be transferred into a carrier (usually *Escherichia coli* K-12) which can synthesize large quantities of the putative protective antigen in vitro (182, 210). However, to induce a cell-mediated response with this protective antigen, it must be presented to the host in some sort of adjuvant and the most effective preparation (Freund complete adjuvant) cannot be used in humans due to its toxicity (40, 197). Even under optimal conditions, a nonreplicating adjuvanted vaccine seems unlikely to induce better levels of protection compared with that achieved by using small numbers of live BCG (49).

LIVE RECOMBINANT DNA VACCINES

Recently, DNA transfers have been achieved by using the nonvirulent vector *M. smegmatis* (116). However, this recombinant vaccine is unlikely to be any more effective than the *E. coli* K-12 protein preparation since the avirulent mycobacterium will be inactivated as soon as it enters the tissues (39). In an attempt to circumvent this limitation, protective genes could be transferred into live BCG or into *M. tuberculosis* H₃₇Ra, both of which are likely to persist in vivo long enough to induce a specific protective cell-mediated immune response. An alternative approach might be the use of vaccinia virus as the carrier. Such attenuated genetically engineered vaccines still carry with them the risk of inducing active disease in hypersusceptible or immunodepleted individuals, and this possibility greatly limits the potential usefulness of these vaccines as vectors in AIDS patients (102).

Another practical consideration which must be borne in mind is the maximum level of protection which any new recombinant vaccine is likely to induce in the host relative to that presently achievable with existing preparations. We know that substrains of BCG can vary substantially in their protective effectiveness in mice depending on the growth and persistence of the organisms in vivo (123). In general, immunity is roughly proportional to the amount of growth which occurs within the spleen of the vaccinated host (40). Thus, to be protective, live recombinant vaccines must produce substantially more protective immunogen in vivo than current BCG vaccines. However, safety considerations mandate that the new recombinant vaccine must not multiply in vivo much more than BCG to avoid the risk of pathological side effects. The best hope is that the new recombinant vaccine will produce large amounts of specific protective *M. tuberculosis* antigen in the absence of competing antigens usually responsible for humoral or suppressor cell responses by conventional BCG vaccine. This same preparation must not be more toxic than BCG vaccine in hypersensitive or hypersusceptible individuals. A number of essential practical problems must be solved before these genetically engineered vaccines can be approved for use in normal adults, let alone in immunodepressed patients and infants.

IMMUNOTHERAPY OF MYCOBACTERIAL INFECTIONS

The development of T-cell cloning techniques makes it theoretically possible to produce large numbers of the cellular mediators of the antituberculous immune response in the laboratory (143, 162). However, the functional activity of

such T-cell clones appears to be surprisingly limited even when infused in large numbers into immunodeficient recipients. This may be because most of these clones were selected by using antigens specific for monoclonal antibodies developed against dead mycobacteria and thus represent helper T cells rather than those involved in the expression of cell-mediated immunity *in vivo* (210). In addition, such *in vitro* prepared cells may not recirculate normally when placed in an *in vivo* environment. As a result, detectable levels of delayed hypersensitivity or acquired antituberculous resistance or both may not be expressed by the recipient host. This problem might be avoided if immune memory T-cell clones are developed since these cells seem to play an instructional rather than a direct effector role in the immune process (146). Development of memory immune T-cell clones to replenish those lost by the immunodepleted host is likely to become an important facet of future attempts to control disseminated infections caused by these chronic intracellular pathogens in AIDS patients.

Demonstration of adoptive immunity to tuberculosis, using cloned cells in the mouse model, requires prior depletion of the T-cell defenses before the infusion of immune T cells into the recipient (148). Experimentally, this type of depletion can be achieved in a number of ways (44). The most commonly used procedure is surgical thymectomy followed by whole-body irradiation and bone marrow reconstitution (Thxb). However, some mature T cells (and their precursors) will be reintroduced into the thymectomized host with the bone marrow cells. For this reason, treatment of the thymectomized host with monoclonal anti-Thy-1.2 antibodies may be preferable. Various monoclonal antibodies can be used to deplete the host of defined T-cell subsets, thereby allowing a better assessment of their role as mediators of tuberculin hypersensitivity, acquired antituberculous resistance, antituberculous memory, suppression, and immune tolerance (157). The number of residual T cells present at the time of mycobacterial challenge will determine the growth rate and survival of the mycobacteria depending on the virulence of the test organism (53). For instance, congenitally athymic (*nu/nu*) mice infected with virulent *M. avium* undergo a rapidly fatal infection, whereas their immunocompetent (*nu/+*) littermates survive such a challenge virtually indefinitely. Conventionally thymectomized (Thxb) mice infected with the same strain of *M. avium* develop a substantially slower and generally nonlethal infection, presumably reflecting the presence of residual T cells in the Thxb host (T. Takashima and F. M. Collins, unpublished data). Despite difficulties in the interpretation of some of these protection studies, the T-cell-depleted host has considerable potential for the development of immunotherapeutic protocols which may eventually protect ARC patients against life-threatening mycobacterioses that may develop later in their disease (19, 78).

M. TUBERCULOSIS INFECTIONS AND AIDS

The AIDS epidemic in the United States dates from two reports published in 1981 stating that several previously healthy homosexual males living in New York, N.Y., and San Francisco, Calif., were dying from a rare form of pneumonia caused by *Pneumocystis carinii*, as well as from a particularly aggressive form of Kaposi's sarcoma (99, 115). Since then, the number of AIDS patients reported to the Centers for Disease Control in Atlanta, Ga., has doubled every year and currently stands at more than 68,000 (Fig. 3), with as many as 250,000 patients projected by the year 1991

FIG. 3. Number of patients suffering with AIDS reported to the Centers for Disease Control each year (●), together with mortality (■) and MAC infections (▲) estimated from an overall 55% death and 30% infection rate (45). Open symbols represent predicted values for 1989.

(59, 80). At the same time, the number of AIDS patients in Africa and South America may reach 10 times this number (160).

Although most AIDS patients die as a result of a *P. carinii* pneumonia (8, 77), many of them have also been reported to be infected with acid-fast bacilli (103, 214). Initially, most of these mycobacterial infections were recognized only at postmortem examination, and it seems likely that many more infections were missed due to the need for specialized staining and cultural methods to detect the presence of these organisms within the tissues (119, 122). In general, only a few American AIDS patients were found to be suffering from *M. tuberculosis* infections, although those who did tended to develop the miliary form of this disease (111, 164, 190). Some of these patients were known to have an earlier history of pulmonary tuberculosis, suggesting that the HIV infection was reactivating dormant lesions previously held in check by the immune T-cell defenses (162). Haitian and African AIDS patients were more likely to develop disseminated *M. tuberculosis* infections, presumably because most of them had been exposed to tuberculosis during childhood (124, 132, 166). Because of this, it is now recommended that all high-risk and HIV antibody-positive Haitians and Africans be routinely checked for tuberculin hypersensitivity and that buffy coats cells be screened for acid-fast bacilli by culture (161). If *M. tuberculosis* is recovered from an HIV antibody-positive individual, this finding should be considered diagnostic for clinical AIDS and the patient should be treated

immediately and aggressively with a multiple tuberculocidal drug regimen (16, 162).

Tuberculosis often develops in these patients in an aberrant extrapulmonary form involving the intestines (and their associated lymphoid tissues), the liver, spleen, kidneys, central nervous system, joints, bone, and bone marrow (15, 25, 30, 159). A further indication of the profound immunosuppression characteristic of these patients is the fact that even the attenuated strain of *M. bovis*, BCG, can give rise to a progressive mycobacteriosis (23). The latter finding has important implications for the use of live BCG (or any of the new recombinant vaccines) in HIV-positive patients who may be at risk of developing tuberculosis. Far from enhancing the level of antituberculosis resistance in these individuals, use of such vaccines may simply drive the ARC patient into the terminal phase of the disease (125). Even the use of recombinant vaccinia virus bearing protective *M. tuberculosis* genes would seem to be contraindicated, since several AIDS patients who were vaccinated during their induction into the armed services have developed severe disseminated vaccinia viremias (170).

One interesting finding to emerge from these studies has been the sharp discrepancy between the mycobacterial isolates recovered from Haitian and American AIDS patients living in Miami, Fla., and New York, N.Y. (104, 163). In one study, 27 of 45 (60%) Haitian AIDS patients were found to be infected with *M. tuberculosis*, whereas only 1 of 37 (3%) American AIDS patients living in the same community were positive for this organism (162). Almost 80% of Haitians are tuberculin positive by the age of 20 (166), and almost one-third of the AIDS patients were reported to be suffering from pulmonary tuberculosis before developing AIDS. Much the same seems to be true for African AIDS patients living in Europe (7, 124, 159). On the other hand, only about 10% of the mycobacterial isolates obtained from American AIDS patients have been identified as *M. tuberculosis* (163). This lower isolation rate seems compatible with tuberculin conversion rates known to occur in this country (183). However, recent epidemiological data suggest that pulmonary tuberculosis is once again on the rise in this country (29), and so *M. tuberculosis* isolates from American AIDS patients may become increasingly common (24, 25).

The majority of *M. tuberculosis*-infected AIDS patients respond quite well to conventional antituberculous chemotherapy (10, 212). Thus, a regimen of streptomycin, isoniazid, and rifampin or ethambutol seems to be highly effective for most of them (25, 27). However, an increasing number of *M. tuberculosis* isolates from Haitian and African patients are drug resistant, requiring the additional use of ansamycin, amikacin, and pyrazinamide in the treatment regimen (163). Furthermore, because of their immunosuppressed state, these patients will have to be maintained indefinitely on an antituberculous drug regimen to prevent recurrence of active disease (159).

NONTUBERCULOUS MYCOBACTERIAL INFECTIONS AND AIDS

Most opportunistic mycobacteria probably enter the tissues across the bronchial or the intestinal mucosae. The low invasiveness characteristic of most of these infections may be due to their inability to compete effectively with members of the normal intestinal flora at the mucosal surface. It is surprisingly difficult to infect normal mice or guinea pigs with mycobacteria by the oral route of challenge (42; Collins and Montalbino, unpublished data). Even when the innately

susceptible C57BL/6 mouse is used, systemic disease develops slowly (Fig. 2) and only then if the organisms are suspended in skimmed milk containing 5% sodium bicarbonate solution. Uptake of mycobacteria following oral administration may be more efficient when carried out prior to weaning or when the host has been pretreated with oral antibiotics (32, 53). However, even closely related mycobacterial species can exhibit wide variations in mouse virulence when introduced by the oral route, and it is difficult to predict the outcome of any specific infection study (39).

The disease pattern seen in many MAC-infected patients seems consistent with an intestinal rather than a respiratory infection pathway (61, 106, 205). Thus, massive Peyer's patch and mesenteric lymph node involvement is not uncommon in these patients, along with considerable intestinal erosion and the development of a chronic diarrhea (16, 174). Why only a limited number of nontuberculous mycobacterial species can be recovered from these severely immunosuppressed AIDS patients is presently unclear (42), but presumably reflects the ability of only a few of these species to colonize the intestinal mucosal surfaces of normal adults. Mouse infection studies suggest that the mucosal defenses may be unable to inactivate certain opportunistic mycobacterial pathogens once they gain entry to the tissues, and so these species tend to be present more frequently in the partially immunodepleted host compared with less persistent strains (73, 84).

A number of AIDS patients have been reported to develop a bizarre and unexpectedly severe mycobacteriosis of the type previously seen only in a few cancer and transplantation patients (8, 77). However, as many as 50% of AIDS patients may be infected with acid-fast bacilli at some time during their disease (42). Species and serological identification of these organisms is a technically demanding procedure which has been attempted only for a relatively small proportion of isolates (112, 120, 212). Of those tested, almost 70% were reported to be MAC serotypes 4 and 8, with only 15% of the typable strains being allocated to the other 25 serovars (95). At least 30% of the isolates were untypable by the standard seroagglutination method, although studies of the glycolipid makeup of these strains suggest that most of them fall into serotype 4 (112, 134, 178). About 5% of AIDS patients develop life-threatening disseminated disease as a result of infection with MAC serovars 4 and 8 (114). The virtual absence of the other virulent MAC serotypes (types 2, 6, 9, 12, 14, and 16, for instance) from the American AIDS population is surprising, given their known presence in other patients living within the same communities (95, 96, 134). About 10% of the mycobacterial isolates have been identified as *M. kansasii* (mostly coming from AIDS patients living in the midwest), together with smaller numbers (6 to 9%) of *M. chelonae*, *M. scrofulaceum*, *M. gordonae*, and *M. fortuitum* (95). Surprisingly, there have been no reports of *M. simiae*, *M. nonchromogenicum*, *M. terrae*, or *M. gastri* isolates from these patients despite their occasional recovery in specimens taken from other patients living in the same areas (96). The reason for the limited number of mycobacterial species recovered from AIDS patients living in this country is something of a mystery, but it does suggest some sort of causal relationship between MAC serotypes 4 and 8 and the development of clinical AIDS in many HIV-infected individuals (42, 125).

It is often difficult to assess the true pathological significance of the opportunistic mycobacteria recovered from many of these AIDS patients (117, 121). Published case reports often give little indication of the extent of any

mycobacterial involvement, stating only that "acid-fast bacilli were present" or "mycobacteria were cultured from biopsy material" (60, 76). The number of acid-fast bacilli present in these patients varied from only an occasional acid-fast rod seen in a single tissue section to uncountable numbers of mycobacteria in blood and biopsy specimens. Since 70% of AIDS patients will die as a result of *P. carinii* pneumonia, the contribution of mycobacterial infections to this process may be quite minimal in many instances. Approximately 5% of AIDS patients develop disseminated mycobacterioses which are directly responsible for the death of the host (114). Death is probably due to a progressive loss of pulmonary function, although acute liver and kidney failure as a result of local heavy mycobacterial involvement in these organs may contribute to this effect (76, 106, 141).

M. avium may have a predilection for the intestinal mucosa of the normal host, suggesting the presence of specific cell wall adhesins (virulence antigens) reactive with receptors on the mucosal cell surface, providing them with a preferential portal of entry into the tissues (131, 193, 201). Colonization of the mucosal membrane by large numbers of these organisms is likely to increase their rate of transfection into the submucosa and the Peyer's patches. The more virulent the MAC serotype, the more likely it is to survive within the GALT tissues of the normal host and thus be present when the T-cell defenses are depleted by the HIV infection (39, 42). Less virulent serovars may also possess the necessary cell wall adhesin but will not survive within the GALT organs. In general, those organisms which can establish themselves on the mucosal surface of the immunodepleted ARC patient will induce an increasingly severe local tissue destruction and a persistent diarrhea (9). The latter may resemble cholera in its intensity, and the lesions in the bowel wall may be similar to those observed in Whipple's disease in humans (205) or in Johne's disease in cattle (32). Interestingly, *M. paratuberculosis* (Johne's bacillus) appears to be a nutritionally demanding strain of *M. avium* (11), suggesting that MAC members may generally be able to induce localized infections within the intestinal tract, although few of these local infections go on to involve the deeper tissues (174). Cultural and serological identification of ultra-slow-growing mycobacteria isolated from these lesions is a technically demanding chore which has been attempted for only a few of these clinical isolates, mostly coming from patients suffering with chronic colitis (33). Specific DNA probes now being developed for these mycobacteria should help to expedite future studies with these technically demanding organisms (208).

A few AIDS patients develop primary rectocolonic mycobacterial lesions; these organisms rapidly seed the local lymphoid tissues, ultimately infecting the spleen and lung, with uniformly fatal results (91). Direct rectal transfer of nontuberculous mycobacteria may occur in some homosexual AIDS patients (60), an infection pathway rendered all the more plausible by the presence of other intestinal parasites in the resulting lesions (22, 92). In many cases, these intestinal mycobacterial infections become so severe that malabsorption and nutritional deficiencies also develop, further lowering host resistance to the infection (173, 205). These organisms can reach enormous numbers within the tissues before they induce detectable clinical, bacteriological, or endoscopic evidence of their presence (66, 120). Furthermore, by virtue of the intermittent nature of these infections, detection of mycobacteria within the intestinal tract can be a technically difficult procedure, partly due to the large number of non-acid-fast contaminants likely to be

present in the specimen (185). Decontamination and cultural methods used to detect these organisms in feces have greatly improved in recent years, and as many as 30% of normal fecal samples are now known to yield isolates of *M. avium* (168). This finding suggests that colonization of the intestinal mucosa by these environmental mycobacteria may be far more common and pervasive than previously believed (101) and may help to explain the presence of *M. avium* in so many ARC and AIDS patients (120, 173, 174).

A few AIDS patients appear to develop primary MAC infections involving only the lung, presumably as a result of bronchial colonization by aerosolized water-borne organisms (68, 156). These patients may show no intestinal involvement (133, 188) although eventually the lung infection will seed into the bloodstream to involve the GALT system. Blood cultures prepared from such patients may be the only effective means of diagnosis since these lung infections seldom result in cavitory disease (14, 61). Blood cultures also provide a useful means for monitoring the effectiveness of any chemotherapeutic regimen since the blood will be rendered culture negative long before the systemic infection begins to decline (10, 206).

African AIDS patients have not been subjected to the same detailed microbiological investigation as their American and European counterparts (169). As a result, we have relatively little information regarding the extent of mycobacterial involvement in the African AIDS patient (124). Most of the available data deal with Africans being treated in Europe for AIDS (7), but it appears that there is a preponderance of *M. tuberculosis* isolates in this group (159). More recent data suggest an increasing proportion of MAC serotype 4 and 8 infections in European AIDS patients (93), and it has been suggested that the more virulent MAC serovars are somehow involved in the development of clinical AIDS in many of these patients also (124).

The nature of the relationship between MAC and HIV infection has been difficult to establish due to lack of acceptable experimental models for either infectious agent (45, 80, 137). The only rodent model presently available for studying MAC infections seems to be the C57BL/6 mouse and its beige mutant, both of which develop progressive systemic infections when challenged orally or aerogenically with *M. avium* or *M. intracellulare* (53, 88). The pace of the resulting systemic infection is relatively dilatory, and the host may die from old age rather than from the mycobacterial infection (42). Normal individuals may become sensitized to the antigens of avirulent mycobacteria if sufficient organisms are ingested over a prolonged period (20). With more virulent (persistent) strains, this initial reactivity may be followed by a persistent state of anergy as a heavy infectious load becomes established within the GALT organs. The eventual result may be a persistent state of immune tolerance which could interfere with the expression of cellular immunity to any subsequent systemic infection caused by this organism in the HIV-infected individual (42). This potentiation is more likely to occur as the T4/T8 cell ratio is reduced below some arbitrary threshold level, when even avirulent species such as *M. gordonae* and *M. fortuitum* (known to be present in some water supplies in substantial quantities) may produce local lesions within the GALT organs (36, 68, 184). However, these infections seldom progress to the point where the impaired cellular defenses are unable to limit their systemic spread to other organs, thus allowing a life-threatening mycobacteriosis to develop. We need to learn a great deal more about the host-parasite

interactions involved after these less virulent mycobacteria enter the immunodepleted tissues (79).

Because of the profound immunodepression which develops in the AIDS patient, the presence of even a few acid-fast bacilli within lung or intestinal biopsy specimens should be accorded the utmost pathological significance (60, 206, 212). Early detection of MAC involvement in the ARC patient is a technically difficult problem for the diagnostic bacteriologist (24, 69, 165, 188), and most of the available tests are slow, tedious, time-consuming, and expensive (122). In many patients, cultivation of buffy coat or bone marrow cells for mycobacteria may provide the only effective diagnostic procedure (18, 100, 167). We desperately need more sensitive and rapid detection methods for specific mycobacterial antigens and their antibodies within the tissues. While a number of radioimmune, enzyme-linked immunosorbent, and DNA relatedness assays have been developed in recent years, we still need to improve their sensitivities and specificities if we are to detect the presence of minute amounts of specific antigen (equivalent to $<10^4$ bacilli per g of tissue) in feces, urine, serum, and bronchial alveolar lavage fluids (102, 175). Such tests are probably several years away, although recent advances in molecular biology will eventually provide the type of highly sensitive methodologies needed to detect these slow-growing opportunistic human pathogens before they become firmly established within the tissues (3).

Similar technical limitations are likely to be involved in the development of more sensitive and discriminatory skin test diagnostics, especially for use in MAC-infected patients (55, 62, 70). Such skin tests can be very difficult to interpret, especially since most AIDS patients are likely to be profoundly anergic as a result of the HIV infection (126). There is a relatively poor degree of cross-responsiveness between the various specific tuberculins (PPD-S, PPD-Y, PPD-A, and PPD-B), and in some cases specific sensitins may be difficult to obtain commercially (55, 70). Most opportunistic mycobacteria are poor skin sensitizers, and any responsiveness is likely to be lost relatively quickly (54). Paradoxically, heavily MAC-infected patients may develop substantial humoral responses to the mycobacterial antigens and the patient may develop severe antigen-antibody complex disease (72, 202). When this response is combined with the strong B-cell mitogenic effect of the HIV infection, some patients may develop an immunological defect similar to that seen in systemic lupus erythematosus (81).

Finally, the MAC infection may itself contribute to the increasing immunosuppression of the HIV-infected patient, thereby helping to drive the disease into its terminal stage (42). Many mycobacterial infections are known to affect T-cell responsiveness, the nontuberculous disease taking on many of the immunological characteristics of lepromatous leprosy (35, 181). However, in these complex infection states it is often difficult to decide which is the horse and which is the cart. Thus, we know that the T4 cell population is depleted by the HIV infection (80) and some of the enhanced microbial growth seen in the AIDS patient may be a direct result of this HIV-induced T-cell depletion.

In nude mice, markedly increased growth by some *M. intracellulare* strains occurs compared with that in their immunocompetent littermates (Fig. 4). Growth of virulent serovars is significantly enhanced in the immunodeficient mouse ($P < 0.01$) and growth continues within the lungs and spleen until all of the mice succumb to the infection (53), whereas the immunocompetent controls survive indefinitely (Takashima and Collins, unpublished data). Such growth

FIG. 4. Growth of BCG 1011 and *M. intracellulare* 673, 1405, and 1406 in the lungs (▲, ▲) and spleens (■, ■) of athymic nude (broken lines) or normal (solid lines) BALB/c mice following aerogenic challenge.

enhancement did not occur when the nude mice were infected with an avirulent strain (TMC 1406) of *M. intracellulare*, the rate of decline in viability being little different from that seen in the immunocompetent controls (Fig. 4). These in vivo growth data appear to be consistent with those reported earlier (42, 197).

As the MAC infection in the HIV immunodepleted host reaches a critical size, the antigenic stimulus within the GALT system may deflect an already limited T-cell resource into an essentially nonprotective humoral response (45). At the same time, this antigenic stimulus increases the number of activated T cells and macrophages available for the HIV to parasitize. Paradoxically, this MAC infection increases the level of immunosuppression within the host tissues without helping the host to develop the type of immune response needed to control the intracellular infection (78, 80). If these assumptions are true, then the MAC infection is itself an additional causal factor of this disease (125, 126). There appears to be a hierarchy among the nontuberculous mycobacteria recovered from these AIDS patients, which presumably is related to the size and persistence of the helper T-cell response they engender. Further investigation of this matter is needed before the role played by these secondary opportunistic pathogens in the evolution of the final stages of this important new human immunological disease can be fully understood.

The prognosis for the MAC-infected AIDS patient is very poor, largely due to the resistance of these organisms to most of the available antituberculous drugs and to the high toxicity of many of these compounds when used at clinically useful levels (10, 120, 212). New mycobactericidal drugs and improved treatment regimens are urgently needed for these patients since even complex mixtures of presently available drugs given at their maximally tolerated doses seem to have little effect on the progress of the disseminated disease (77).

Some clinical improvement has been reported in a few patients treated with ansamycin, clofazimine, ethambutol, amikacin, and ethionamide, but for the most part MAC-infected AIDS patients remain untreatable (56, 66). At best, the drugs are bacteriostatic only, with mycobacterial growth being resumed whenever treatment has to be curtailed due to toxicity (81, 125). For this reason, new chemotherapeutic and immunotherapeutic agents will have to be developed for use in these patients. So far, all attempts to use bone marrow transplants, T-cell clones, recombinant interleukin-2, and gamma interferon infusions have proven clinically ineffective (19). Clearly, a great deal of innovative research is needed to develop a more effective type of therapy for these heavily MAC-infected AIDS patients (78, 79, 102).

SUMMARY AND CONCLUSIONS

The genus *Mycobacterium* contains several important human and animal pathogens, including *M. tuberculosis*, *M. bovis*, *M. kansasii*, and *M. avium*. However, this group also contains many environmental saprophytes which bear an uncertain relationship to human disease. After nearly a century of intensive study, we still know very little about the factors responsible for the pathogenicity of the tubercle bacillus or how they assist the organism to survive within the body. Some nontuberculous mycobacteria have been recognized as potential human pathogens only since the widespread introduction of chemotherapeutic drugs and immunosuppressants. A few reports of disseminated disease due to *M. kansasii*, *M. avium*, and *M. intracellulare* can be found in the early tuberculosis literature, but the incidence of these infections has increased sharply with the emergence of the AIDS epidemic.

Colonization of normal mucosal membranes by environmental mycobacterial species may be relatively common in many parts of the world (especially in tropical and subtropical regions). The more virulent of these opportunistic mycobacteria may become virtual members of the commensal gut and nasopharyngeal flora, surviving within the submucosal layers for relatively long periods of time. Colonized individuals become skin test positive to *M. avium* and *M. intracellulare* sensitins. Systemic involvement by these organisms occurs only when the normal T-cell defenses are depleted as a result of old age, radiation, chemotherapy, or an intercurrent HIV infection. The latter group of AIDS patients seem especially prone to disseminated MAC infections, suggesting the existence of some sort of causal relationship between these two phenomena. Once established within the tissues, the MAC infection may involve virtually every organ of the body (liver, spleen, kidney, central nervous system, bone marrow, and intestinal tract). The source of most of these infections is presumed to be the environment, but it has proven very difficult to demonstrate the actual infection pathway, and whether the MAC infection precedes or follows HIV exposure has yet to be established definitively.

Up to 50% of AIDS patients develop a mycobacterial infection at some stage of their disease. Two-thirds of the isolates are MAC serotypes 4 and 8, 10% are *M. tuberculosis*, 8% are *M. kansasii*, and 6% are *M. scrofulaceum*. Many of these isolates show aberrant cultural characteristics, colony pigmentation, mycobacteriophage susceptibility, plasmid content, and drug resistance. The significance of these differences is presently unclear.

Large numbers of *M. avium* (up to 10^{10} acid-fast bacilli per g of tissue) have been reported in some AIDS patients. This

heavy tissue load, combined with the high drug resistance of these organisms, makes effective treatment of these patients almost an impossibility. As a result, the AIDS epidemic represents a return to the prechemotherapy era for patients with systemic MAC disease, and we urgently need to develop new drugs and better treatment regimens for use in this rapidly expanding patient population.

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