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Health Impacts of Environmental Mycobacteria†

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INTRODUCTION

There have been a number of excellent reviews on the clinical presentations and treatment of nontuberculous mycobacteria (4, 38, 86, 88, 122). This review aims to give a broader view of the health impacts of interactions between humans and environmental mycobacteria and discuss a number of factors which relate to those interactions. The environmental mycobacteria (also called atypical mycobacteria, nontuberculous mycobacteria, or mycobacteria other than tuberculosis) (32) are a fascinating group of human, animal, and bird pathogens. They have significant impacts on the morbidity and mortality of humans and important economic impacts on agriculture.

There are currently 91 identified species in the genus *Mycobacterium* not in the *M. tuberculosis* complex (37). In spite of the recent profusion of new mycobacterial species, recent reports document that 30% of mycobacterial isolates from water, soil, air, and patients do not belong to any of the identified species (115). Likely there are numbers of species yet to be discovered.

ENVIRONMENTAL OPPORTUNISTIC MYCOBACTERIA

Environmental opportunistic mycobacteria are distinguished from the members of the *M. tuberculosis* complex (and *M. leprae*) by the fact that they are not obligate pathogens but are true inhabitants of the environment. They can be found as saprophytes, commensals, and symbionts. Environmental mycobacteria include both slow-growing (i.e., colony formation requires 7 days or more) and rapidly growing (i.e., colony formation in less than 7 days) species. It should be noted that rapidly growing mycobacteria still grow significantly more slowly than most bacteria. In fact, based upon differences in 16S rRNA gene sequences, the slowly and rapidly growing mycobacteria could be split into two different genera (110). Environmental mycobacteria exhibit great variation in growth rates (2- to 48-h doubling times), colony morphologies (29, 128), antibiotic and biocide sensitivities (21), plasmid carriage (31, 59, 89), and virulence (29). Shared characteristics of environmental mycobacteria (along with the *M. tuberculosis* complex) are great hardiness, an acid-fast cell wall containing mycolates, and intracellular pathogenicity.

ENVIRONMENTAL RESERVOIRS

Locations

Environmental mycobacteria are normal inhabitants of a wide variety of environmental reservoirs, including natural and municipal water, soil, aerosols, protozoans, animals, and humans (see Table 1). Either external association with or outright invasion of plants is also a potential (33, 69). Water is likely the primary source of *M. avium* complex infection in humans (44), though not the only source (116). DNA-based fingerprints of *M. avium* isolates from AIDS patients were identical to those of isolates recovered from the patients' drinking water (118). Further, DNA fingerprints of *M. avium* isolates from simian

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† This article is dedicated to the memory of F.M.P.

TABLE 1. Environmental isolations of mycobacteria[a]

Species[b]	Location	Reference(s)
Unknown	Scots pine tissue	69
M. gordonae, MAC	City aquarium, drinking water	44
MAC	Residential water sources (i.e. toilet, tap, shower)	116
MAC	Hospital recirculating hot water system	118
MAC	Numerous municipal potable water sources	7
M. fortuitum	Sewage treatment plant	10
M. scrofulaceum, MAC, *M. szulgai*, *M. fortuitum*, & others	Surface water (Rio Grande)	15
M. fortuitum	Soil (in Malawi)	17
MAC	Soil	18
M. scrofulaceum, MAC, *M. szulgai*, *M. fortuitum*, *M. gordonae*, & *M. simiae*	Hospital tap water (in Taiwan)	22
M. flavescens, *M. austroafricanum*, *M. chlorophenolicum*, & unknown	Petroleum-contaminated soil	23
M. mucogenicum, *M. kansasii*, *M. gordonae*, MAC, *M. fortuitum*, & others	Public drinking and potable water sources, ice machines, water treatment plant	30
MAC	Hot tubs	35
M. terrae, MAC, & *M. scrofulaceum*	Water-damaged buildings in Finland	52, 54
MAC, *M. gordonae*, *M. fortuitum*, & *M. kansasii*	Public swimming pools and whirlpools	47
M. immunogenum	Biocide-treated metalworking fluid	66, 80, 121, 125
M. chelonae	Gentian violet solution	97
Many species	Domestic and wild animals, numerous species	9
M. xenopi & *M. botniense*	Natural surface water streams in Finland	114
M. marinum, *M. chelonae*, *M. gordonae*, *M. fortuitum*, & others	Public swimming pools in Italy	72
M. ulcerans	Natural waters, soil, insects, wild animals, fish	91
MAC	Water and soil of brown water swamps	60

[a] This table is meant to be illustrative not all inclusive and thus not every publication is included.
[b] Unknown, 16S ribosomal sequence did not match any known species. MAC, *M. avium* complex.

immunodeficiency virus-infected macaques were identical to those from the sole source of drinking water for the monkeys (74).

The prevalence of many species of environmental mycobacteria in municipal drinking water supplies (40) is directly explained by their high innate chlorine and biocide resistance (70, 112). Treatment of a pilot water system with ozone or chlorine resulted in a dramatic shift in the bacterial population to the *Actinomyces* family, which includes *Mycobacterium* (83). Furthermore, environmental mycobacteria are capable of biofilm formation (*M. fortuitum* and *M. chelonae* [45], and *M. avium* [personal communication, F. Quinn]) and thus mycobacterial populations can persist in a flowing system (e.g., water distribution system) in spite of their slow growth.

Environmental mycobacteria also have extraordinary starvation survival (84, 107), persisting despite low nutrient levels in tap water. *M. intracellulare* persisted with only one log loss of viability after 1.4 years in deionized sterile water (6). Furthermore, tolerance of temperature extremes (102) results in contamination of hot tap water, spas, and ice machines by environmental mycobacteria, with *M. avium* complex, *M. xenopi*, *M. phlei*, and *M. chelonae* being the most thermoresistant species. *M. mucogenicum*, *M. kansasii*, *M. gordonae*, and *M. flavescens* are among the many other species of environmental mycobacteria isolated from public potable water (30, 67, 71). Food is also a source of human exposure to environmental mycobacteria, as mycobacteria were present in 25 of 121 food samples (20%), and certain isolates of *M. avium* showed genetic homology to clinical isolates (129).

Large numbers of mycobacteria, including *M. avium* complex, *M. gordonae*, *M. malmoense*, *M. simiae*, and *M. marinum*, are found in *Sphagnum* vegetation and in peat-rich soils and waters of Finland (57, 101, 119), as well as acidic, brown water swamps of the eastern United States (60). It is likely that the high percentage of *M. avium* infection among Finnish AIDS patients is a consequence of the large number of *M. avium* in water (96). A variety of physiological characteristics of *M. avium* and *M. intracellulare* contribute to their large numbers in those environments. *M. avium* and *M. intracellulare* have an acidic pH optimum for growth between 4.5 and 5.5 (43), and their growth is stimulated by humic and fulvic acids, the major organics in peat and the brown water swamps (61). Larger numbers of *M. avium* and *M. intracellulare* have been recovered from waters and soils of low oxygen levels (18, 60), and representatives of these species can grow at microaerobic conditions (Lewis and Falkinham, unpublished data). Thus, the mycobacteria are ideally suited for life in those environments. Furthermore, adaptations to acid and microaerobic conditions aid in the virulence of intracellular pathogens.

Consequences of Overlapping Human and Mycobacterial Ecology

There are a variety of situations where human and mycobacterial geographic and environmental distributions can overlap and lead to exposure of humans as well as impacting mycobacterial ecology. A major overlap occurs with water. Humans are exposed to mycobacteria in water through drinking, swimming, and bathing. Aerosols generated during these activities can also lead to human exposure. Cervical lymphadenitis in children is hypothesized to be a result of mycobacteria in drinking water and possibly from soil which contaminates dirty objects placed in the mouth. The presence of environmental mycobacteria in water coupled with their disinfectant

TABLE 2. Double-edged mycobacterial sword

Drawback	Feature	Advantage
Slow growth	Single rRNA cistron	Antimicrobial resistance Adaptation
Impermeable	Wax-rich cell wall	Antimicrobial resistance
Transport limit		Hydrocarbon permeable
Energy demand of fatty acid synthesis		General stress tolerance
Impermeable to hydrophilic nutrients	Hydrophobic surface	Resistance to hydrophilic antimicrobials Surface attachment Concentration at air-water interface Readily aerosolized Readily phagocytosed

resistance leads to the presence of environmental mycobacteria in hot tubs, solutions used in medical treatment, e.g., gentian violet (97), and water-oil emulsions used to cool metalworking tools (80, 125). Dusts can be rich sources of environmental mycobacteria, especially dust rich in peat. Foods and cigarettes may also be sources of mycobacterial infection.

Human activities are likely to influence the distribution and prevalence of mycobacteria. First, treatment of drinking water supplies with chlorine or other disinfectants (e.g., ozone) leads to selection for environmental mycobacteria (83). While *M. avium* complex exhibits higher chlorine resistance than other species (94; unpublished data from Primm and Falkinham), even the weaker species such as *M. aurum* are 100-fold more tolerant than *Escherichia coli* (70).

Environmental mycobacteria present in drinking water supplies and not removed by treatments such as turbidity reduction are less affected by disinfection than other bacteria. That, coupled with the ability of environmental mycobacteria to grow in natural waters containing even low concentrations of organic matter, leads to mycobacterial predominance in drinking waters. In addition, differences in the chlorine susceptibility of different mycobacterial species may alter the mycobacterial species profile of drinking water. For example, before 1970, the majority of cases of cervical lymphadenitis in children were caused by *Mycobacterium scrofulaceum* (127). Since 1975, *M. avium* has been the predominant species isolated (126). *M. scrofulaceum* is fivefold more sensitive to chlorine than is *M. avium* (J. Falkinham, unpublished data). Implementation of clean water acts in the United States that increased chlorination rates starting in 1975 may have led to a strong reduction of *M. scrofulaceum* in water.

PHYSIOLOGICAL ECOLOGY

The physiological ecology of environmental mycobacteria refers to the identification of physiological characteristics of environmental mycobacteria that are determinants of their ecology and hence epidemiology. These important characteristics of the slow-growing environmental mycobacteria are presented in Table 2. Slow growth of mycobacteria is due to the possession of either one (slow growers) or two (rapid growers, except *M. chelonae* and *M. abscessus*, which have only one) (92) 16S rRNA cistrons (*E. coli* has seven operons), impermeability of the lipid-rich cell wall, and the synthetic energy cost of the long-chain mycolic acids (e.g., C_{60} to C_{90}). While the possession of a single rRNA cistron constrains mycobacteria to their characteristic slow growth, it also grants them greater ease of accumulating a resistance mutation for ribosomal-targeting antibiotics. The lower metabolic rate of slow growth also imparts more time for adaptation in stressful environments.

The impermeability of the cell wall is not necessarily a disadvantage because it endows mycobacteria with innate resistance to a wide range of antimicrobial agents, including antibiotics and disinfectants (e.g., chlorine). It also plays a major role in intracellular survival during infections of animals and protozoans, an important part of the mycobacterial life cycle. The complex lipid-rich cell wall, granting the property of acid fastness, also results in a hydrophobic cell surface that is a major determinant of environmental distribution.

Environmental mycobacteria are found at air-water interfaces where complex and hydrophobic hydrocarbons are found and enriched relative to bulk water. Environmental mycobacteria can metabolize a wide range of these hydrophobic hydrocarbons, including a number of chlorinated hydrocarbon pollutants (10, 23, 94, 123, 124). The importance of hydrocarbon utilization is supported by the unusually large number of genes involved in lipid catabolism in the genomes of mycobacteria, approximately five times that of *E. coli* K-12 (19, 27). The concentration of both bacilli and the hydrocarbon nutrient sources at the interface of an aqueous environment are of obvious benefit.

Hydrophobicity is also a factor leading to binding of environmental mycobacteria and association with particulate matter. Mycobacterial numbers correlate with raw water turbidity in drinking water treatment plants (40). Reduction of raw water particulate content reduces mycobacterial numbers in treated water. Thus, the innate hydrophobicity of environmental mycobacteria attracts the most likely nutrient sources of particulates and small organic compounds. Environmental mycobacteria possibly attach to surfaces simply by hydrophobic interactions, and may be biofilm "pioneers" (45). Biofilm formation on pipes is a major survival factor in municipal water systems. Hydrophobic bacilli are more readily aerosolized, and aerosols are a major delivery mechanism for environmental mycobacteria to obtain pulmonary access to animal hosts.

Thus, the same physiological factors which slow the growth and restrict the nutrient access of mycobacteria also grant tremendous compound and stress tolerance and provide favorable hydrophobic interactions facilitating nutrient acquisition, biofilm formation, and spread by aerosolization. In fact, phylogenetic analysis of ribosomal sequences suggests that slow growth is of recent evolution in mycobacteria and, as discussed above, possibly of great adaptive value (90).

PROTOZOAN INTERACTIONS

Interactions of environmental mycobacteria with protozoans are very important for a number of reasons. Many protozoans are bacterial grazers, and the ability to survive phagocytosis by protozoans is a considerable advantage to water-borne bacilli. *M. avium*, *M. fortuitum*, and *M. marinum* all invaded and replicated inside *Acanthamoeba*, while the soil-dwelling *M. smeg-*

matis was killed (26). *M. avium* inhibits lysosomal fusion and possibly kills infected amoebae. *M. avium* can also invade and replicate in *Dictyostelium discoideum* (106). Compared to bacilli grown in medium, amoeba-grown *M. avium* are more invasive towards amoebae or human epithelial and macrophage cells (26). Amoeba-borne intracellular *M. avium* are more invasive towards mouse intestine, and thus protozoans may serve as vectors during oral transmission of environmental mycobacteria.

These intracellular bacilli also exhibit enhanced resistance to antibacterials (78). *M. avium* grown in *Tetrahymena pyriformis* are more virulent in chickens than those grown in laboratory medium (39). *M. avium* can also grow on compounds released by *Acanthamoeba polyphaga* (111), and *T. pyriformis* cells infected with *M. avium* grow more rapidly than uninfected *T. pyriformis* (J. Falkinham, unpublished data), suggesting an exchange of compounds during cogrowth. Thus, environmental mycobacteria exhibit parasitic and symbiotic relationships with protozoans.

Intracellular *M. avium* can survive during encystment and be released upon excystment (111), thus potentially using protozoan cysts as carriers to survive starvation and toxic stresses. We used protozoa and amoebae to isolate mycobacteria from water (J. Falkinham, unpublished data). The protozoa and amoebae were grown to starvation and added to water samples (raw surface water). After 1 week of incubation, the protozoa or amoebae were isolated by low-speed centrifugation and mycobacteria were isolated by spreading the concentrates on M7H10. Preliminary results indicate different species of mycobacteria from the protozoa compared to sampling the water directly.

We hypothesize that protozoans have played a central role in the evolution of mycobacterial pathogenesis. Selection of mycobacteria that can infect and replicate in protozoans has likely resulted in mycobacteria also becoming intracellular pathogens in animals. In support of this, *Legionella pneumophila*, which shares the characteristics of aquatic life, protozoan infection, and intracellular pathogenicity, uses highly overlapping gene sets to replicate in human macrophages and acanthamoebae (103).

ILLUSTRATIVE CASE STUDIES

Two categories of infections caused by environmental mycobacteria will be examined briefly in order to illustrate a number of principles discussed previously.

Cervical Lymphadenitis in Children

Mycobacteria have long been known as one agent responsible for cervical lymphadenitis in children. The age of children in the majority of cases is 6 months to 2 years and coincides with the period of time that teeth are erupting. Infection is limited to cervical and mandibular lymph nodes. Swelling of the lymph nodes is usually the first evidence of infection, although a draining sinus can result if the infection is untreated. Antimycobacterial therapy is of little efficacy, and surgical removal of the infected lymph nodes has had the best prognosis for cure.

It is likely that children serve as sentinels for the presence of mycobacteria in water. The species shift in *Mycobacterium* causing cervical lymphadenitis in children is not just in the United States, but has also been reported in the United Kingdom (28) and Australia (75, 77; R. Dawson, personal communication). Because it is unlikely that children have changed, it is likely that the change is due to the change in the prevalence of *M. scrofulaceum* and *M. avium* in the water.

Recently it was observed in Sweden that the incidence of cervical lymphadenitis in children caused by environmental mycobacteria rose dramatically after the cessation of BCG vaccination of children (56). Evidently, vaccination protected children against cervical lymph node infection by environmental mycobacteria.

Aerosol-Associated Infections

Recently there have been reports of hypersensitivity pneumonitis as a consequence of exposure to aerosols that contain mycobacteria. These reports include workers in occupational situations (e.g., metal grinding) and individuals at home (e.g., hot tubs and spas). Hypersensitivity pneumonitis has been reported in automobile workers exposed to aerosols generated from metalworking fluid used in metal grinding and finishing operations (66, 80), in life guards exposed to aerosols generated in indoor swimming pools (47), and individuals at home exposed to aerosols from aerated hot tubs (35), spas (55), humidifiers, and water-damaged building materials (120). In a number of instances, mycobacteria, including *M. avium*, *M. chelonae*, and a novel species of *Mycobacterium* (recently named *M. immunogenum* (125), have been recovered from the fluid or water. In all instances, the fluid or water had been subject to disinfection before symptoms appeared in exposed workers or individuals. As with municipal water chlorination, the disinfection procedure selected for the intrinsically resistant mycobacteria. Unless mycobacteria are considered, disinfection procedures, especially addition of biocides to systems, often provide these hardy bacilli an ecological niche.

ROUTES OF INFECTION

Environmental mycobacteria are present in almost every municipal water source (22), and genomic restriction fragment patterns of *M. avium* from hospital water isolates are similar to those from AIDS patient isolates (7). Environmental mycobacteria are also ubiquitous in natural water sources (C. S. Bland, H. Fuller, T. P. Primm, and M. E. Alvarez, 102nd Annu. Meet. Am. Soc. Microbiol., 2002, abstr. Q-392). Water, almost exclusively in piped systems, is a source of *M. kansasii*, with aerosols being involved in transmission (76). *M. xenopi* is unique in that hot water systems, particularly recirculating, are a major source (38). Water is also a source for *M. marinum*, which infects through skin abrasions (1, 36). *M. fortuitum*, *M. chelonae*, and *M. abscessus* are all water borne, with soil a source as well (38).

The source of *M. simiae*, *M. malmoense*, and *M. haemophilum* is still uncertain (122). Members of the *M. avium* complex commonly infect birds, yet symptomatic infection in mammals is rarer and rarely transmissible (113). The primary infection routes are oral and aerosol (93). Although a number of studies have attempted to determine which routes lead to *M. avium* infection in AIDS patients, the need to decontaminate sputum and fecal specimens from AIDS patients reduced the level of sensitivity of detection, so no firm conclusions could be drawn (24, 53). A combination of both routes is likely.

Consistent with an oral route of transmission, *M. avium* exhibits high innate acid (16) and bile salt tolerance (T. Primm, unpublished data). *M. avium* infects the intestinal mucosa at the terminal ileum, primarily through the apical surface of epithelial cells and not via M cells (12, 98, 99). Invasion is enhanced by growth at 37°C versus 30°C, high osmolarity and low oxygen tension, yet unaffected by low pH and iron limitation (11, 13). While environmental mycobacteria are opportunistic pathogens in a variety of immunocompromised patients, the wide prevalence results in all humans being commonly and continuously exposed at low levels (50 to 500 bacilli per day).

We believe that the majority of human-mycobacteria interactions are transient, self-curing colonizations. While the immune systems in the majority of the population clear the bacilli, the resulting release of potent immunomodulators, such as trehalose dimycolate, may generate other consequences not typically attributed to this interaction. Lipids extracted from *M. bovis* BCG recruit immune cells to granuloma in mice (R. Geisel, B. Rhoades, and D. Russell, Tuberculosis Keystone Symposium, Taos, N.Mex, 2003, poster 223; B. Rhoades, R. Geisel, B. Butcher, and D. Russell, Tuberculosis Keystone Symposium, Taos, N.Mex., 2003, poster 244). The most likely candidates for these downstream effects are allergies, pulmonary viral infections, and irritations of the bowel. These subclinical human-mycobacteria interactions may give a transient repression or stimulation of certain immune pathways, setting the stage for other diseases, as discussed below.

Only a very small percentage of human-mycobacteria interaction progress to outright mycobacterial infection, but such progression is much more common in immunocompromised patients, especially those with AIDS (5). In a survey of PPD-B (the Battey strain of *M. intracellulare*) skin test reactivity of 18- to 25-year-old men who were single-county residents, it was shown that greater than 60% of men from the southeastern coastal region of the United States showed positive skin tests (34). Thus, they were infected and produced a detectable immune response to mycobacterial antigens yet did not show any signs of disease. Other studies show similar high environmental mycobacteria skin test reactivity in U.S. medical staff (117), the elderly in Israel (104), and Kenyan children (68).

CRYPTIC RELATIONSHIPS TO OTHER DISEASES

Chronic bowel disease. *M. avium* subsp. *paratuberculosis* causes Johne's disease in ruminants and has been proposed as a cause of Crohn's disease in humans (46, 82). Most but not all patients with Crohn's exhibit significant improvement in response to antimycobacterial therapy (105). This controversy will not be discussed in this review, but environmental mycobacteria causing chronically progressing bowel disease will be considered. *M. avium* subsp. *paratuberculosis* causes inflammation and damage to the intestine in SCID mice, similar to chronic bowel disease (81).

As mentioned previously, *M. avium* invades intestinal tissue, and *M. tuberculosis* can cause intestinal infection as well (2). *M. avium* oral infection of immunocompetent mice results in a strong inflammatory response and necrosis of the intestinal mucosa (58). *M. genavense* infections in human immunodeficiency virus patients often result in abdominal wall thickening, lymphadenopathy, and ulceration (79). The prevalent characteristics of chronic bowel disease (slow progression, excessive inflammatory response, intestinal invasion) fit well with mycobacterial involvement.

Allergies. There has been an increase in allergies and other TH2 autoimmune disorders in the past few decades in Western society, possibly due to the hygiene hypothesis, e.g., that a cleaner environment and fewer childhood infections result in more autoimmunity. Others think changes in diet are the cause. A shift from TH1 to TH2 responses promotes allergy. However, type 1 diabetes and inflammatory bowel disease are TH1 diseases and have also increased. Thus, a simplistic view of enhanced TH2 responses does not explain disease prevalence.

Reduced exposure to environmental mycobacteria may indeed lead to more allergies (14). In mice, BCG vaccination resulted in a TH1 response which lowered airway allergic responses (less immunoglobulin E, eosinophils, interleukin-4, and interleukin-5) to ovalbumin sensitization (48). Vaccination with *M. vaccae* or BCG prevented asthmatic responses, including bronchoconstriction, against ovalbumin in mice (49, 50), even if given after sensitization to ovalbumin. Inoculation of rats with BCG also lowered TH2 responses, including lowered immunoglobulin E and interleukin-4 levels (64, 65). Intranasal application of live or even heat-killed BCG reduced eosinophil numbers and TH2 response to ovalbumin in mice (73).

BCG vaccination is being considered as a therapy for allergies in humans (25). However, oral exposure of mice to *M. vaccae* can either enhance or block BCG skin sensitization, depending on timing or exposure (20). Mycobacteria grown in laboratory culture medium have been shown to provoke allergic responses in cell lines (51, 52). Exposure to environmental mycobacteria also causes hypersensitivity pneumonitis, as mentioned above. It should be noted that hypersensitivity pneumonitis results from inflammatory products released by mycobacteria, not necessarily infection.

Pulmonary viral infections. The strong dysregulation of pulmonary immunity, exemplified by hypersensitivity pneumonitis, would also predispose the affected to succumb to the constant assault of aerial viruses. There is a well-established synergistic interaction during coinfection with *M. tuberculosis* and human immunodeficiency virus, with the cytokine profile elicited by the bacteria stimulating viral entry and replication (75), at least in part due to tumor necrosis factor alpha-mediated transcriptional activation of human immunodeficiency virus long terminal repeats (62). *M. avium*, *M. smegmatis*, and *M. bovis* also stimulate human immunodeficiency virus replication in human cells (63). Orally administered heat-killed *M. phlei* shifted the immune response in chickens against Newcastle virus towards cell-mediated immunity, with a decrease in neutralizing antibodies (109). The protective effect of the immune response was not significantly altered, however.

Glycopeptidolipid from *M. avium* inhibits the lymphocyte blastogenic response to human T-lymphotrophic virus type 1 (77). Conversely, administration of Z-100, an arabinomannan from *M. tuberculosis*, reduced splenomegaly and increased survival of mice infected with LP-BM5 murine leukemia virus

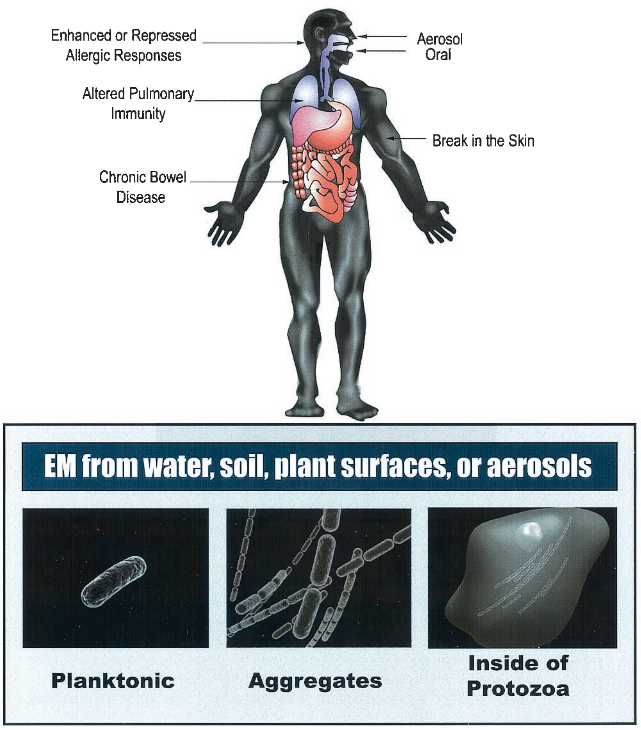

FIG. 1. Complexity of human-mycobacterial interactions. Depending on what physiological state the mycobacteria are in (represented by the lower panel), the immunocompetence of the human, the route of entry of the mycobacteria (represented by the right side of the upper panel), the numbers and virulence of the mycobacteria, which species of environmental mycobacteria (EM), and other factors; human-mycobacteria interaction may result in many different effects on humans (upper panel, left side).

(100). Thus, strongly immunomodulatory cell wall-derived compounds from mycobacteria can affect immune responses against viruses.

Vaccine efficacy. *M. avium*, *M. scrofulaceum*, and *M. vaccae* exposure in mice (2×10^6 CFU, subcutaneous, three infections at 2-week intervals) blocks replication of BCG, preventing vaccine protection against *M. tuberculosis* (17). In contrast, exposure to rapidly growing environmental mycobacteria in Malawi (various unknown doses, likely low, via multiple routes) protected humans against tuberculosis and leprosy (41). As mentioned above, *M. vaccae* infection (oral via drinking water) either enhanced or suppressed BCG skin sensitivity, depending on timing of 0, 24, or 54 days before BCG injection (20). It is well established that there are serious issues of cross-reactivity with human environmental mycobacteria exposure and tuberculosis purified protein derivative skin testing (3, 8, 68, 104, 117). It has been suggested that a major reason for the poor efficacy of BCG vaccination against tuberculosis in the Indian Chingelput trial was common exposure of humans in that area to environmental mycobacteria (108).

The complex effects of environmental mycobacterial interactions with humans resulting in increased or decreased immunity to tuberculosis and leprosy are not yet understood, much less with other pathogens or vaccinations. Thus, depending on the timing, dosage, bacterial state, and route of exposure, environmental mycobacteria may prevent or predispose towards a number of medical conditions (Fig. 1). Note that these conditions would not typically be recognized as a result of human-mycobacteria interaction. Thus, mycobacteria may have far greater effects on humans than the clinically diagnosed mycobacterial infections.

CONCLUSIONS AND PREDICTIONS

We predict an increasing incidence of interactions between humans and mycobacteria in coming years. This will likely result in more clinical cases of environmental mycobacteria. Three major factors driving this increase are (i) disinfection of drinking water with chlorine, selecting mycobacteria by reducing competition, (ii) disinfection attempts in medical and industrial settings may likewise select for mycobacteria, and (iii) the increasing percentage of our population with predisposing conditions, most notably AIDS, age, and immunosuppressive regimens, e.g., after transplantation. We also predict a rebound in the caseload of *M. avium* complex infections in AIDS patients as drug-resistant human immunodeficiency virus inevitably spreads. Whether environmental mycobacteria are a contributor to the increase in autoimmune disorders as well remains to be determined.

Second, novel environmental opportunistic mycobacterial species will continue to be identified. This is driven, in part, by more rapid and sophisticated methods for identification (e.g., 16S rRNA gene sequencing), the increasing number of individuals predisposed to environmental mycobacterial infection, and the increasing use of disinfectants to "sterilize" habitats. From the other angle, humans are having a major impact on mycobacterial ecology. Witness the apparent loss of *M. scrofulaceum* from the environment and its replacement by *M. avium*, quite possibly as a result of widespread chlorination of drinking water.

Research in understanding the physiological ecology of mycobacteria is needed to fully discover the effects that mycobacteria have on humans and to allow us to intervene when necessary. Efforts must be focused on actions that will specifically remove mycobacteria from habitats where humans or animals can be exposed. For example, the discovery that mycobacteria in raw drinking water sources are associated with particulates and that particulate reduction reduces mycobacterial numbers (40) led to the recommendation that mycobacterial numbers can be lowered by reduction of water turbidity. Turbidity reduction has already been mandated by the Environmental Protection Agency in the United States.

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