The Pathogenesis and Treatment of Mycoplasmal Infections

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Summary

Pathogenic mycoplasmas have been found in the blood or other specimens of patients with a variety of chronic clinical conditions, including respiratory, oral cavity, genital and other infections, autoimmune, inflammatory and immunosuppressive diseases and fatigue syndromes of unknown origin. These small bacterial microorganisms are possible causative agents, cofactors or opportunistic infections in these and other illnesses. Evidence for their association or possible role in various clinical conditions is suggested by their significantly higher incidence or degree of infection in symptomatic patients than in non-symptomatic controls and their gradual suppression by the appropriate antibiotics resulting in gradual patient recovery from clinical signs and symptoms. Although they are not widely appreciated for their pathogenic properties, certain *Mycoplasma* species and certain other species of bacteria (*Chlamydia*, *Borrelia*, etc.) appear to play a role in disease progression or patient morbidity in rather large subsets of chronic illness patients.

Introduction

Certain *Mycoplasma* species, the smallest and simplest, free-living, bacteria that lack a rigid cell wall, are important pathogens in animal, plant and insect species. In humans mycoplasmal infections have only recently been associated with certain acute and chronic illnesses where they may function as causative agents, cofactors or opportunistic infections that cause patient morbidity. Although various *Mycoplasma* species are commonly found as commensals in the oral cavity and at other superficial sites, certain species appear to cause morbidity when they penetrate into the blood and spread to and colonize various tissues. For example, *M. hominis* and *Ureaplasma urealyticum* are common inhabitants of the human genital tract but they can play an etiologic role in pyelonephritis, pelvic inflammatory diseases and post-abortion and post-partum fevers. Some reports claim that some *Mycoplasma* species cause serious systemic infections, such as septicemia, septic arthritis, neonatal meningitis and encephalitis, and this has been confirmed in animal models. For example, *M. fermentans* can cause severe, fatal neurological and respiratory signs and symptoms after injection into the cerebral fluid of rats. Although sometimes questioned, several pathogenic *Mycoplasma* species have been proposed to be etiologic agents in various acute and chronic diseases in man. Less appreciated is the possibility that multiple chronic infections, including *Mycoplasma* species, play an important role in various chronic illnesses and their progression.

Mycoplasma genomes are the smallest among bacteria

The genomes of most *Mycoplasma* species encode about 600 proteins. For example, The *M. genitalium* and *M. pneumoniae* genomes contain 470 and 677 protein-coding gene sequences, respectively, compared with 1,703 protein genes in *Haemophilus influenzae* and about 4,000 genes in *E. Coli*. The genomes of *M. genitalium* and *M. pneumoniae* have lost the genes involved in certain biosynthetic pathways, such as the genes for amino and fatty acid and vitamin synthesis. Since they are cell wall-deficient bacteria, there is a major reduction in genetic information needed for cell wall biosynthesis. Although *Mycoplasma* species carry a minimal set of genes involved in energy metabolism and biosynthesis, they still have the essential genes for DNA replication, transcription, translation, and the minimal number of rRNA and tRNA genes. The reduction in mycoplasmal genomes explains their need for host nutritional molecules. A significant number of mycoplasmal genes appear to be devoted to cell adhesion and attachment organelles as well as variable membrane surface antigens to maintain parasitism and evade host immune and nonimmune surveillance systems.

Mycoplasma species variably express structurally heterogeneous cell surface antigens. Variations in the genes encoding cell surface adherence molecules reveal distinct patterns of mutations capable of generating changes in mycoplasma cell surface molecular size and antigenic diversity. Variable surface antigenic structures and rapid changes in their expression are thought to play important roles in the pathogenesis of mycoplasmal infections by providing altered structures for escape from immune responses and protein structures that enhance cell and tissue colonization

Mycoplasma interactions with host immune systems

Certain *Mycoplasma* species can either activate or suppress host immune systems, and they may use these activities to evade host immune responses. For example, some mycoplasmas can inhibit or stimulate the proliferation of normal lymphocyte subsets, induce B-cell differentiation and trigger the secretion of cytokines, including interleukin-1 (IL-1), IL-2, IL-4, IL-6, tumor necrosis factor- (TNF), interferons, and granulocyte macrophage-colony stimulating factor (GM-CSF) from B-cells as well as other cell types. Moreover, it was also found that *M. fermentans*-derived lipids can interfere with the interferon (IFN)-g-dependent expression of MHC class II molecules on macrophages. This suppression results in impaired antigen presentation to helper T-cells in an experimental animal model. Also, mycoplasmas are able to secret soluble factors that can stimulate proliferation or inhibit the growth and differentiation of immune competent cells.

Mycoplasma species are known to secrete immune-modulating substances. For example, immune cells are affected by spiralin, a well-characterized mycoplasmal lipoprotein that can stimulate the in vitro proliferation of human peripheral blood mononuclear cells and murine splenocytes. This stimulation of immune cells results in secretion of proinflammatory cytokines (TNF, IL-1 or -6). Spiralin can also induce the maturation of murine B-cells.

As described above, mycoplasmas can evade immune recognition by undergoing surface antigenic variations thus rapidly altering their cell surface structures. Such antigenic variability, the ability to suppress host immune responses, slow growth rates and intracellular locations may explain the chronic nature of mycoplasmal infections and the common inability of a host to suppress mycoplasmal infections with host immune and nonimmune responses.

Rapid adaptation to host microenvironments by mycoplasmas is usually accompanied by rapid changes in cell surface adhesion receptors for more successful cell binding and entry as well as rapid structural protein changes to mimic host antigenic structures (antigen mimicry). For example, during chronic, active arthritis the size and antigenic diversity of the surface lipoprotein Vaa antigen changes in structure and expression in vivo. Antigenic divergence of Vaa can affect the adherence properties of *M. hominis* and enhance evasion of host-mediated immunity. Variations in the Vaa genes reveal a distinct pattern of mutations that generate mycoplasma surface variations and thus avoid host immune responses.

Mycoplasmas Can Induce Programmed Cell Death and Necrosis

Mycoplasmas can directly suppress host immune responses by initiating or enhancing apoptosis. For example, *M. fermentans*, an AIDS-associated mycoplasma, can initiate or enhance concanavalin A-induced apoptosis of T-cells. Relatively large amounts of nucleases are also expressed by *Mycoplasma* species, and these can be released intracellularly to cause degradation of host DNA. Mycoplasmal nucleases may also be involved in secondary necrosis seen in advanced mycoplasmal infections, as indicated by the occurrence of morphological characteristics of apoptosis (chromatin condensation) and necrosis (loss of membrane integrity and organelle swelling). Although mycoplasmas can release activated oxygen species that may be involved in initiating apoptosis, some *Mycoplasma* species, such as *M. fermentans*, express a novel cytolytic activity in a nonlipid protein fraction that has a cytocidal effect not mediated by the known mycoplasmal cytokines like TNF.

In addition to apoptosis, mycoplasmas can also release growth inhibitory molecules into their surroundings, such as arginine deaminase. This enzyme can act as a growth-inhibitory substance that suppresses IL-2 production and receptor expression in Tcells stimulated by non-specific mitogens, and it can induce the morphologic features of dying cells and DNA fragmentation indicative of apoptosis.

Clinical Testing for Mycoplasmal Infections

Until recently one of the most difficult problems in detecting mycoplasmal infections was that the available techniques, serological and culturing procedures, were relatively insensitive for detecting intracellular infections. Mycoplasma culture techniques can be highly specific for detection of some mycoplasmal infections, but they are relatively insensitive because of difficulty culturing various *Mycoplasma* species. Conventional serological detection of mycoplasmal infections is quite difficult due to the lack of humoral immune responses in most patients. Also, detection methods that use antibodies against mycoplasma antigens are not very reliable, because mycoplasmas are able to hide inside cells. This can result in rather normal antibody titers during active mycoplasmal infections.

The most reliable clinical testing for mycoplasmal infections uses whole blood, blood leukocytes or tissue biopsies and polymerase chain reaction (PCR). Even with this approach it is necessary to insure that intracellular *Mycoplasma* species are being detected at high sensitivity. Another research technique that has been used for intracellular infections is nucleoprotein gene tracking. This approach detects mycoplasmal genes directly in

nucleoprotein complexes isolated directly from cell nuclear fractions. Although highly specific, it is not as sensitive as PCR.

Persistence of Mycoplasmal Infections and Various Clinical Conditions

Mycoplasmas have been found at significantly higher incidence in blood and tissue specimens obtained from patients with various chronic illnesses compared to healthy controls. Since little is known about the involvement of mycoplasmas in the pathogenesis of chronic illnesses, it remains uncertain whether these findings indicate that some *Mycoplasma* species are causal agents, cofactors, or opportunistic (superinfections) in patients with immundisturbances. Since mycoplasmas can be found at superficial sites, such as normal flora in the genitourinary tract, oral cavity and gut where they are thought to be nonpathogenic. The distinguishing characteristic in pathogenic infections may be the penetration of *Mycoplasma* species into the blood circulation and especially into cells in various tissues. This may explain the finding of pathogenic *Mycoplasma* species in genitourinary tract, oral cavity, gut and the blood in a few percent of asymptomatic subjects. Unless mycoplasmas penetrate into tissues and cells, it is unlikely that they can exert their pathogenic effects, but in some individuals the presence of mycoplasmas is not associated with any clinical condition. In such cases it is not apparent whether this represents a superficial infection, an early nonsymptomatic or dormant phase of the illness process or a carrier phenomenon.

The persistence of mycoplasmal infections has many similarities with Chlamydial persistance. Certain *Chlamydia* species infections can remain dormant and do not always progress to replication and host cell lysis, and similarly certain *Mycoplasma* species can remain inside cells for long periods without initiating apoptosis and eventual cell lysis. Unlike *Chlamydia* species where much is known about the dormant or cryptic intracellular phase of their life cycles, little is known about the mechanism of persistence of mycoplasmal infections. Both of these bacteria (at least their pathogenic strains) are considered obligatory intracellular parasites because they are dependent on host cell intermediary metabolites and biosynthetic precursors, and they are thought to cause much of their pathogenic effects during their intracellular persistance phase. Alternatively, when intracellular pathogens, such as certain *Mycoplasma* species, are released from cells without cell lysis, they can carry host cell surface antigens with them, eventually resulting in autoimmune host responses against the infected tissues.

Mycoplasmal Infections and Respiratory Illnesses

Various respiratory illnesses, such as chronic asthma, airway inflammation, chronic pneumonia and other respiratory diseases, are known to be associated with mycoplasmal infections. For example, *M. pneumoniae* is a common cause of upper respiratory infections, and severe asthma is commonly associated with mycoplasmal infections. Recent evidence has shown that certain mycoplasmas, such as *M. fermentans* (incognitus strain), are unusually invasive and found within respiratory epithelial cells. Similar to certain *Chlamydia* species, pulmonary macrophages appear unable to kill pathogenic *Mycoplasma* species.

Although mycoplasmal infections are often associated with chronic asthma, the exact role of mycoplasmas in the pathogenesis of asthma remains unclear. Certain *Mycoplasma* species are involved in respiratory tract infections associated with airway inflammations, induction of bronchial hyperresponsiveness (BHR) and asthmatic attacks. At a minimum, *M. pneumoniae* infections can cause worsening of conditions in asthmatic patients, whose attacks are associated with significant and specific IgA and IgE responses. Specific antibodies of these subclasses for *M. pneumoniae* protein antigens were found in a majority of patients with *M. pneumoniae* infections. Mycoplasmas are only one of many agents that can trigger BHR, and other infectious or chemical agents may contribute to the complex disease process.

Mycoplasmal Infections in Urogenital Diseases

Mycoplasma species are commonly found in urogenital infections. For example, *M. hominis* was detected in more than 12% of females who presented at gynecological services, and *M. genitalium* has been associated with acute and nonspecific non-gonococcal urethritis in males but not in asymptomatic controls. This organism is also a common cause of genital infections in women, and it was detectable in 7% of women with sexually transmitted diseases. *M. hominis* and *U. urealyticum* have been implicated in a wide variety of urogenital diseases, such as pelvic inflammatory disease, infertility, non-gonococcal urethritis (NGU) and other genital infections, pyelonephritis, Reiter's syndrome, and peritonitis. The appearance of various bacterial species in bacterial vaginosis may be a result of pathophysiological alterations of the vaginal ecosystem, and mycoplasmas appear to play an important role in this process. Mycoplasmas are also known to interfere in pregnancy, For example, *U. urealyticum* was found to be involved in 11% of patients with fertility problems.

Some *Mycoplasma* species, *M. fermentans*, *M. penetrans*, and *M. pirum,* have been implicated as infectious cofactors in HIV-AIDS. Using relatively insensitive techniques all three mycoplasmas have been detected in up to 20% of patients with HIV infections, and serological studies have suggested that the presence of *M. penetrans* is also associated with HIV infection. Moreover, the incidence of systemic mycoplasmal infections in HIV-AIDS patients could be much higher than previously thought. Most of the analyses were performed using relatively insensitive techniques, such as serological analysis. Pathogenic *Mycoplasma* species may influence HIV pathogenesis by specific and direct activation or suppression of the immune system, the production of superantigens with subsequent alterations in immune responses, or their contribution to the oxidative stress observed in HIV-positive patients. Also, the development of AIDS may increase the susceptibility of HIV-infected patients for coinfection with various *Mycoplasma* species, such as *M. fermentans*. This species is able to bind HIV capsid protein gp120 permitting adhesion of HIV virions to the mycoplasma surface. Subsequently the HIV viruses could be transported directly to cells expressing CD4 receptors. After binding to target cells, mycoplasmas can stimulate host cell activation by IL-1 and TNF, which are known effectors for virus reproduction. In addition, oligosaccharides of the mycoplasmal glycocalyx may protect bound HIV-1 virons from host immune responses.

Antigen similarities between the surface components of mycoplasmas and HIV-1 have led to speculation that they use similar mechanisms for cell entry. For example, the HIV1 gp120 envelope glycoprotein and *M. genitalium* adhesion proteins share sequence homology and also have significant similarity with the CD4-binding site of the class II major histocompatibility complex (MHC) proteins. The interactions of microorganisms with MHC-related antigens on host cells could contribute to a number of possible outcomes, including T-cell dysfunction, T-cell depletion, T-cell shift, B-cell proliferation, hyperglobulinemia and antigen-presenting cell dysfunction. Interestingly, all of these have been observed during the development of HIV-AIDS.

Mycoplasmal Infections in Rheumatic Diseases

Although the underlying causes of rheumatic diseases are not known, rheumatoid arthritis (RA) and other rheumatic illnesses may involve, at least in part, infectious agents. In addition, the progression of rheumatic diseases may also be related to infectious processes. The remarkable clinical and pathological similarities between certain infectious diseases in some animal species and those of some human rheumatic illnesses, such as RA, have encouraged the search for microbial etiologies for these syndromes. A long list of microorganisms, including aerobic and anaerobic intestinal bacteria, several viruses and *Mycoplasma* species have been proposed as important in these illnesses. We recently found multiple mycoplasma species in about one-half of the blood samples from RA patients using PCR. All multiple infections occurred as combinations of *M. fermentans* with other species.

Mycoplasma species are known to be able to induce immundysfunction and autoimmune reactions that could be related to the development of RA. In animal models of RA, *M. arthritidis*-related superantigens were found to compromise T-cells, and they can trigger and exacerbate autoimmune arthritis. Furthermore, *M. arthritidis* can release substances that can act on polymorphnuclear granulocytes, such as oxygen radicals and chemotactic and aggregating substances. Also, the isolated membranes of *M. arthritidis* possessed toxic effects when injected into various animals.

Mycoplasmal Infections in Cardiac Diseases

Mycoplasmal infections of the heart have been reported in patients with different types of carditis. The most common association was with *M. pneumoniae* infection. Endocarditis and myocarditis associated with *M. pneumoniae* infections appear to be an important cause of death in *M. pneumoniae* infections. Direct bacterial invasion of *M. pneumoniae* into pericardial tissue appears to be more likely to cause pericarditis than autoimmune phenomena. Viral and bacterial (*Mycoplasma*, *Chlamydia* and *Mycobacterium tuberculosis*) infections appear to be common causes of myocarditis and/or pericarditis, and this is just beginning to be appreciated by infectious diesase specialists.

Mycoplasmal Infections in Autoimmune Diseases

Although pathogenic mechanisms have not been established in autoimmune diseases, mycoplasmal infections seem to play an important but not well understood role in these diseases. Several characteristics of mycoplasmas make them attractive as agents that may be responsible for triggering autoimmune responses. First, during their intracellular replication and release from host cells mycoplasmas can capture antigens from the host cell surface and incorporate them into their cell membranes. This can lead to immune responses against these antigens and possibly autoimmune reactions. Second, mycoplasmal antigens can mimic host antigens and trigger immune responses against these antigens with resulting cross reactivity against host antigens. Third, mycoplasmas can cause apoptosis of host cells with subsequent release of normal host antigens.

Superantigens are potent immunomodulators derived from microorganisms, such as bacteria, viruses and

mycoplasmas. Their effects on immune systems are the result of their binding both to MHC-binding sites on antigen presenting cells and binding to structures within hypervariable regions of Tcell antigen receptors. The contributions of microbial superantigens to the pathogenesis of autoimmune diseases have been investigated in experimental animal models where a superantigen, the mycoplasma arthritis Tcell mitogen, was arthritogenic in mice. When injected into mice, *M. arthritidis* causes a chronic arthritis that resembles RA in its pathology and pathogenesis. Mycoplasmal infections have also been implicated in the progression of Kawasaki disease, Graves' disease, Hashimoto's disease, Sjögren's syndrome, systemic lupus erythematosis (SLE) and multiple sclerosis (MS).

Mycoplasmal Infections in Fatigue Illnesses

Chronic fatigue is the most commonly reported medical complaint of all patients seeking medical care. However, the fatigue syndromes, such as chronic fatigue syndrome (CFS, sometimes called myalgic encephalomyelitis), fibromyalgia syndrome (FMS) and Gulf War illnesses (GWI) are distinguishable as separate syndromes that have muscle and overall fatigue as major characteristics, among many other multiorgan signs and symptoms, including immune system abnormalities. Because of the complex nature of these illnesses, many patients are often diagnosed with multiple syndromes. We and others have examined the presence of mycoplasmal blood infections in CFS, FMS and GWI patients and have found that the majority of patients have blood mycoplasmal infections.

Patients with CFS or FMS often have multiple mycoplasmal infections and probably other chronic infections as well. When we examined CFS/FMS patients for the presence of *M. fermentans*, *M. pneumoniae, M. penetrans, M. hominis* infections, multiple infections were found in over one-half of 93 patients. CFS/FMS patients had double (>30%) or triple (>20%) mycoplasmal infections, but only when one of the species was *M. fermentans* or *M. pneumoniae* (17). We also found higher score values for increases in the severity of signs and symptoms in CFS/FMS patients with multiple infections. CFS/FMS patients with multiple mycoplasmal infections generally had a longer history of illness, suggesting that patients may have contracted additional infections during their chronic illnesses.

Antimicrobial Therapy for Mycoplasmal Infections

Once mycoplasmal infections have been identified in subsets of chronic illness patients, they can be successfully treated, if the therapy continues for some time to eliminate or suppress dormant forms of the microorganism. Using this strategy appropriate treatment with antibiotics can result in patient improvement and even recovery. The recommended treatments for diagnosed mycoplasmal blood infections require long-term antibiotic therapy, usually multiple 6-week cycles of doxycycline (200-300 mg/day), ciprofloxacin (1,500 mg/day), azithromycin (500 mg/day) or clarithromycin (750-1,000 mg/day). Multiple cycles are required, because few patients recover after only a few cycles, possibly because of the intracellular locations of mycoplasmas like *M. fermentans* and *M. penetrans*, the slow-growing nature of these microorganisms and their ability to exhibit persistence as dormant forms and their relative drug sensitivities. For example, of 87 GWI patients that tested positive for mycoplasmal infections, all patients relapsed after the first 6-week cycle of antibiotic therapy, but after up to 6 cycles of therapy 69/87 patients recovered and returned to active duty. The clinical responses that were seen were not due to placebo effects, because administration of some antibiotics, such as penicillins, resulted in patients becoming more not less symptomatic, and they were not due to immunosuppressive effects that can occur with some of the recommended antibiotics.

Chronic illness patients often have nutritional and vitamin deficiencies that must be corrected. These patients are often depleted in vitamins B, C and E and certain minerals. Unfortunately, patients with these chronic illnesses often have poor absorption. Therefore, high doses of some vitamins must be used, and others, such as vitamin B complex, must be given sublingual. Antibiotics that deplete normal gut bacteria can result in over-growth of less desirable flora, so *Lactobacillus acidophillus* supplementation is recommended. In addition, a number of natural remedies that boost the immune system are available and are potentially useful, especially during antibiotic therapy or after therapy has been completed. They appear to be useful during therapy to boost the immune system or after antibiotic therapy in a maintenance program to prevent relapses.

Conclusions

Why aren't physicians successfully treating mycoplasmal, chlamydial and other chronic infections? In many cases they are treating these infections, but they are often not taking into account the intracellular persistent phases of these infections. And it has been only recently that such infections have been found in so many unexplained chronic illnesses. These infections cannot be successfully treated with the usual short courses of antibiotics due to their intracellular locations, slow proliferation rates, persistence and inherent insensitivity to most antibiotics. In addition, a fully functional immune system may be essential to overcoming these infections, and this is why vitamin and nutritional supplements are important in the therapy. Finally, chronic illness patients must be weaned off

antidepressants and other potentially immune suppressing drugs before they can fully recover from their illnesses.

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