Introduction to Mycoplasma

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Description of the Organism

- Prokaryotes
- That lack a cell wall
- Bounded by a cell membrane containing sterol
- Sterols, substances not found in other bacteria or viruses.
- Small size (150 to 250 nm)
- Deformable membrane
- Able to pass through filters with pore sizes that retain other bacteria.

Description of the Organism

- When first discovered, they were thought to be viruses
- Grow in cell-free medium
- They contain both RNA and DNA
- The above two properties set them apart from this class of microorganisms
- DNA homology, failed to demonstrate a significant relationship between mycoplasmas and known bacteria
- Mycoplasmas probably devolved from gram-positive bacteria through reductive evolution

- They require many exogenous nutrients for growth, including vitamins, amino acids, nucleic acid precursors and, in particular, lipids.
- Lipids are provided by the addition of serum or cholesterol to growth medium
- Energy is supplied by carbohydrate metabolism
- Some nonfermenting mycoplasmas derive energy from amino acid (arginine) metabolism

- Most mycoplasmas grown on agar form colonies with a dense central zone and a less dense peripheral zone.
- The resultant colony has been likened to the shape of a fried egg

Taxonomy and Distribution

- Mycoplasmas have now been assigned taxonomically to their own class, Mollicutes
- The family Mycoplasmataceae is composed of two genera responsible for human infection, Mycoplasma and Ureaplasma
- The genus Mycoplasma has more than 13 species that infect humans

Pathogenesis

- Mycoplasmas appear to cause infection primarily as extracellular parasites.
- They attach to the surface of ciliated and nonciliated epithelial cells.
- M. pneumoniae, M. penetrans, and M. genitalium, have special attachment organelles containing adhesin molecules
- M. fermentans and M. hominis, do not have special attachment organelles although they effectively penetrate cells

- The lipid-associated membrane proteins present on the mycoplasmal surface are recognized by Toll-like receptors on cells
- Interraction between these entities may provoke an inflammatory response to the organism
- Subsequent events are unclear but may include direct cytotoxicity of such elaborated substances as hydrogen peroxide,
- Or they may involve cytolysis via an inflammatory response mediated through chemotaxis of mononuclear cells
- upregulation or downregulation of inflammatory cytokines, or antigen-antibody reactions.

- Mycoplasma organisms are very common contaminants of tissue cultures.
- In these cases, they are most often intracellular parasites.
- This fact may contribute to the difficulty in eradicating mycoplasmas from contaminated cultures
- Their presence has been shown to markedly alter cellular and viral molecular events, a fact that has prompted some to question many of the molecular biologic results derived from tissue culture experiments

Defining Characteristics of Mycoplasmas and Ureaplasmas

General

- Prokaryotic
- Small size: 150-250 nm
- No cell wall
- Trilayered cell membrane
- Most are aerobic
- Fastidious growth requirements
- Form fried egg colonies on agar

Differentiation from bacteria

- L-forms Sterols in membrane
- No DNA homology with known bacteria
- Low guanine + cytosine content
- Low-molecular-weight genome (580 to ~2200 kb)
- No reversion to walled forms.

Differentiation from viruses

- Contain both DNA and RNA
- Free-living—cell
- Free growth on defined media
- In vitro Extracellular
- Parasitism in vivo

UNCOMMON MYCOPLASMA ASSOCIATED WITH HUMAN DISEASE

- Mycoplasma incognitus (a variant of M. fermentans), Mycoplasma penetrans, and Mycoplasma pirum have been associated with severe disease in healthy people
- It also causes diseases in Acquired immunodeficiency syndrome (AIDS) patients
- M. fermentans was first isolated by Lo and colleagues from the blood, organs, and Kaposi's sarcoma lesions of patients with AIDS
- Has been reported to cause fulminant multisystem infection in presumably healthy patients

- Mycoplasma found in humans are usually susceptible in vitro to tetracycline, chloramphenicol, clindamycin, and the quinolones
- Sensitivity to the macrolides appears to be very limited.

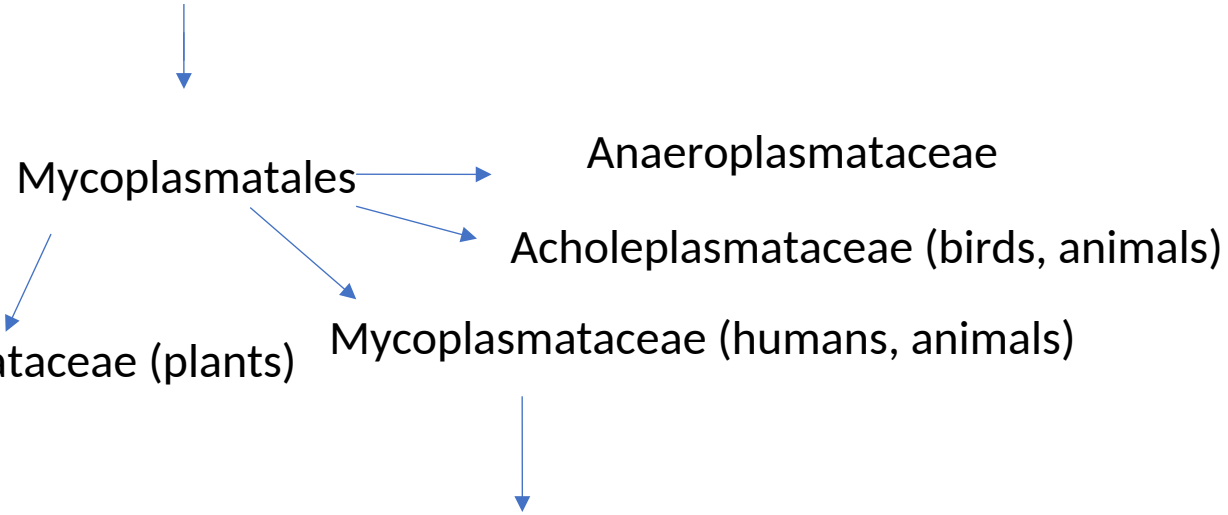
ANIMAL MYCOPLASMA ORGANISMS AS HUMAN PATHOGENS

- Case reports have recently appeared of human infections caused by mycoplasmas previously thought to infect only animals
- Fatal septicemia involving M. arginini (a commensal in cattle, sheep, and goats) occurred in an immunocompromised slaughterhouse worker

GENITAL MYCOPLASMA ORGANISMS CAUSING NONGENITAL INFECTION

- A number of reports have indicated that the genital mycoplasmas (Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum and U. parvum) can cause serious infections involving the respiratory tract, heart, bloodstream, central nervous system, sternotomy wounds, and prosthetic valves and joints of infants and adult

- Class: Mollicutes
- Order



Subgroup Sites of isolation Occurrence Diseases

Genus: Mycoplasma (human)

Subgroup	Sites of isolation	Occurrence	Diseases
M. Hominis	GU tract (F>M)	Common	Cervicitis, vaginitis, ? prostatitis
	Conjunctiva (neonate)		Conjunctivitis
	Blood (peripartum)		Peripartum sepsis
	Surgical wounds, joints		Sternotomy infection, arthritis, especially in immunocompromised

• M. orale	Oropharynx	Common	?
• M. pneumoniae	Respiratory tract	Common	URI, pneumonia
• M. buccale	Oropharynx, gingiva	Common?	Periodontal disease
• M. faucium	Oropharynx	Uncommon	?
• M. fermentans	GU tract	Uncommon	
	Blood, tissues	Uncommon	? Fulminant multisystem disease in patients with AIDS and healthy individuals

Mycoplasma pneumoniae and Atypical Pneumonia

- The concept of atypical pneumonia arose at the onset of the antibiotic era. In the early 1940s, sulfonamides and then penicillins were introduced into clinical practice. At that time, it was recognized that some cases of pneumonia did not respond to these antibiotics and that these were the pneumonias that could not be attributed by Gram stain or culture to a known bacterial cause. The condition was designated primary atypical pneumonia (PAP). The prefix primary indicated that no causative agent could be determined.

- In the intervening years, with the advent of virology and better techniques for identifying fastidious bacterial and protozoan agents, it has become clear that the atypical pneumonia syndrome can be caused by influenza virus, adenovirus, respiratory syncytial virus, cytomegalovirus, Chlamydophila sp., Legionella sp., Pneumocystis jirovecii, Mycoplasma pneumoniae, the newly described coronavirus variant causing severe acute respiratory syndrome (SARS), and probably numerous other agents

History

- In 1945, Eaton and colleagues described an agent that passed through virus filters and caused focal areas of pneumonia when inoculated in several species of rodents. The agent, initially thought to be a virus, could be serially passaged in chick embryos but could not be grown in culture. The relation of this agent to the PAP syndrome was suggested by the fact that human serum from some patients recovering from PAP neutralized the agent. Serum from about 50% to 70% of these patients also was found by Finland and colleagues to agglutinate red blood cells when a mixture of the two was exposed to the cold (4°C). When serum from patients with PAP caused by a known etiologic agent (e.g., influenza virus) was used, cold agglutinins were not demonstrable, and there was no neutralization of Eaton agent. This provided a link between Eaton agent and a proportion of PAP cases of unknown cause. However, serum from this same group of patients also had antibodies to Streptococcus sp. This and other nonspecific antibody formation in these patients served to confuse matters for a time and to detract from the evidence that Eaton agent was a major cause of PAP.

- Because Eaton agent passed through virologic filters and could be grown only in chick embryos, it was believed throughout most of two decades after its discovery that the agent was a virus. In the early 1960s, it was established that the organism had many characteristics in common with those that caused pneumonia in cattle, hence the transiently used term pleuropneumonia-like organism. These organisms were soon shown to be mycoplasmas of the class Mollicutes

Description of the Organism

- M. pneumoniae is a short rod (about 10 × 200 nm)
- Has at one end an organelle that is responsible for attachment of the organism to cell membranes
- The major adhesion proteins of this organelle (P1, P30, P116, and HMW1-3) have been identified and confer on M. pneumoniae its affinity for respiratory epithelium
- Actions of these adhesion proteins are primarily responsible for the organism’s pathogenesis
- M. pneumoniae is prokaryotic and has a very small genome (about 800,000 base pairs)

- The organism is bounded by a trilamellar membrane containing sterols.
- It divides by binary fission, with a doubling time of more than 6 hours.
- 6 This long doubling time makes culturing of M. pneumoniae a slow process (5 to 20 days), compared with bacteria.
- Because they lack a cell wall, mycoplasmas including M. pneumoniae are not affected by β-lactam antibiotics and are not visible on Gram staining.

Transmission

- M. pneumoniae infection is spread from one patient to another by respiratory droplets produced by coughing
- Relatively close association with the index case appears to be required.
- The disease is usually introduced into families by a young child, and in some studies, most of the adults who were infected were the parents of young children.
- Mycoplasma has an incubation period of 2 to 3 weeks.
- Organisms can be cultured from the sputum of infected individuals for weeks to months after clinically effective treatment

Clinical Disease

- Specific, confirmed diagnosis of this entity is not often accomplished in routine clinical practice
- There are probably four reasons for this.
- The first is that mycoplasma pneumonia is usually self-limited and rarely fatal. This fact dampens the zeal to establish the cause of infection.
- Second, mycoplasmas are relatively fastidious and slow growing; therefore, culture results, if obtained at all, often return after the patient is well.
- Third, M. pneumoniae responds to the empirical antimicrobial therapy suggested for CAP, and
- Finally, there is deficient knowledge of the epidemiology and clinical manifestations of infection, so that the diagnosis is often not considered.

Pathology and Pathophysiology

- Because of two fortunate aspects of mycoplasma pneumonia—its low severity and low mortality—there is relatively little information on pathologic findings in this disease, and knowledge rests on relatively few specimens.
- Sickle cell disease, sickle-related hemoglobinopathies, and hypogammaglobulinemia predispose to increased severity and to mortality.
- Some of the available pathologic data therefore may be influenced by the pathophysiology of these underlying conditions.

Diagnosis

- Culture of the organism from respiratory secretions or body fluids should be the gold standard for diagnosis
- However, mycoplasmas are fastidious in their growth requirements, and culture of M. pneumoniae is an elaborate and time-consuming procedure requiring specialized media
- Because of this and the relative infrequency of requests for culture, most hospital microbiology laboratories are not set up to culture mycoplasmas
- Further identification can be established by other growth characteristics and by specific direct immunofluorescence.

Identifying Properties of Mycoplasma pneumoniae

- Slow growth on cell-free media
- Both aerobic and anaerobic growth “Mulberry” rather than “fried-egg” colonies
- Ferments glucose as major nutritional source, producing acid
- Hemadsorption to colonies
- Hemolysis by hydrogen peroxide
- Affinity for respiratory epithelium
- Infection leads to cold agglutinin formation
- Resistance to cell wall inhibitors
- Inhibited by macrolides, tetracyclines, and quinolones

Treatment

- Recommended standard therapy for mycoplasmal pneumonia in teenagers and adults would include doxycycline, 100 mg every 12 hours, or an extended-spectrum macrolide such as azithromycin, 500 mg on day 1, and then 250 mg every 24 hours.
- The usual duration of therapy is 7 to 14 days.
- Young children should be given erythromycin, 10 mg/kg every 6 hours, or an extended spectrum macrolide (azithromycin), 10 to 12 mg/kg on day 1, followed by 5 mg/kg daily for 10 to 14 days.
- Infection of extrapulmonary sites may require prolonged treatment at higher doses.

Prevention

- Because of outbreaks of M. pneumoniae respiratory infection among military recruits, there was for a time great enthusiasm and activity to produce a vaccine to protect against this organism. The vaccines did induce specific antibody responses, but protection against infection was limited to no more than 50% of vaccine recipients.
- Live vaccines using attenuated wild-type and temperature-sensitive mutant mycoplasma have proved no more effective

Genital Mycoplasmas: Mycoplasma genitalium, Mycoplasma hominis, and Ureaplasma Species

- The microbial flora of the human genital tract is complex, including organisms that are difficult to cultivate and detect and most likely organisms that have yet to be discovered.
- Mycoplasmas and ureaplasmas fit into the difficult-to-grow category.
- To date, eight species of mycoplasmas and ureaplasmas have been identified in the genital tract

- Although six species are classified in the genus Mycoplasma, they are far more heterogeneous than their classification implies.
- Mycoplasma genitalium has the smallest genome of any mycoplasma (580 kb) and is closely related to Mycoplasma pneumoniae despite its much larger genome (816 kb).