Mycoplasmas - Stealth Pathogens

By Leslie Taylor, ND January, 2001

Mycoplasmas are a specific and unique species of bacteria - the smallest free-living organism known on the planet. The primary differences between mycoplasmas and other bacteria is that bacteria have a solid cell-wall structure and they can grow in the simplest culture media. Mycoplasmas however, do not have a cell wall, and like a tiny jellyfish with a pliable membrane, can take on many different shapes which make them difficult to identify, even under a high powered electron microscope. Mycoplasmas can also be very hard to culture in the laboratory and are often missed as pathogenic causes of diseases for this reason.

The accepted name was chosen because Mycoplasmas were observed to have a fungi-like structure (Mycology is the study of fungi - hence "Myco") and it also had a flowing plasma-like structure without a cell wall - hence "plasma". The first strains were isolated from cattle with arthritis and pleuro-pneumonia in 1898 at the Pasteur Institute. The first human strain was isolated in 1932 from an abscessed wound. The first connection between mycoplasmas and rheumatoid diseases was made in 1939 by Drs. Swift and Brown. Unfortunately, mycoplasmas didn't become part of the medical school curriculum until the late 1950's when one specific strain was identified and proven to be the cause of atypical pneumonia, and named Mycoplasma pneumonia. The association between immunodeficiency and autoimmune disorders with mycoplasmas was first reported in the mid 1970s in patients with primary hypogammaglobulinemia (an autoimmune disease) and infection with four species of mycoplasma that had localized in joint tissue. Since that time, scientific testing methodologies have made critical technological progress and along with it, more mycoplasma species have been identified and recorded in animals, humans and even plants.

While Mycoplasma pneumonia is certainly not the only species causing disease in humans, it makes for a good example of how this stealth pathogen can move out of it's typical environment and into other parts of the body and begin causing other diseases. While residing in the respiratory tract and lungs, Mycoplasma pneumonia remains an important cause of pneumonia and other airway disorders, such as tracheobronchitis, pharyngitis and asthma. When this stealth pathogen hitches a ride to other parts of the body, it is associated with non-pulmonary manifestations, such as blood, skin, joint, central nervous system, liver, pancreas, and cardiovascular syndromes and disorders. Even as far back as 1983, doctors at Yale noted:

"Over the past 20 years the annual number of reports on extrapulmonary symptoms during Mycoplasma (M.) pneumoniae disease has increased. Clinical and epidemiological data indicate that symptoms from the skin and mucous membranes, from the central nervous system, from the heart, and perhaps from other organs as well are not quite uncommon manifestations of M. pneumoniae disease." (15)

This single stealth pathogen has been discovered in the urogenital tract of patients suffering from inflammatory pelvic disease, urethritis, and other urinary tract diseases (8) It has been discovered in the heart tissues and fluid of patients suffering from carditis, pericarditis, tachycardia, hemolytic anemia, and other coronary heart diseases. (9, 10, 14) It has been found in the cerebrospinal fluid of patients with meningitis and encephalitis, seizures, ALS, Alzheimer's and other central nervous system infections, diseases and disorders. (11-13) It has even been found regularly in the bone marrow of children with leukemia. (16-18) It is amazing that one single tiny bacteria can be the cause of so many seemingly unrelated diseases in humans. But as with all mycoplasma species, the disease is directly related to where the mycoplasma resides in the body and which cells in the body it attaches to or invades.

Today, over 100 documented species of mycoplasmas have been recorded to cause various diseases in humans, animals, and plants. Mycoplasma pneumonia as well as at least 7 other mycoplasma species have now been linked as a direct cause or
significant co-factor to many chronic diseases including, rheumatoid arthritis, Alzheimer's, multiple sclerosis, fibromyalgia, chronic fatigue, diabetes, Crohn's Disease, ALS, nongonoccal urethritis, asthma, lupus, infertility, AIDS and certain cancers and leukemia, just to name a few.\(^{(1-6)}\)

In 1997, the National Center for Infectious Diseases, Centers for Disease Control and Prevention's journal, Emerging Infectious Diseases, published the article, **Mycoplasmas: Sophisticated, Reemerging, and Burdened by Their Notoriety**, by Drs. Baseman and Tully who stated:

"Nonetheless, mycoplasmas by themselves can cause acute and chronic diseases at multiple sites with wide-ranging complications and have been implicated as cofactors in disease. Recently, mycoplasmas have been linked as a cofactor to AIDS pathogenesis and to malignant transformation, chromosomal aberrations, the Gulf War Syndrome, and other unexplained and complex illnesses, including chronic fatigue syndrome, Crohn's disease, and various arthritides."

Mycoplasmas, unlike viruses, can grow in tissue fluids (blood, joint, heart, chest and spinal fluids) and can grow inside any living tissue cell without killing the cells, as most normal bacteria and viruses will do. Mycoplasmas are frequently found in the oral and genito-urinary tracts of normal healthy people and are found to infect females four times more often than males, which just happens to be the same incidence rate in rheumatoid arthritis, fibromyalgia, Chronic Fatigue and other related disorders.\(^{(7)}\)

Mycoplasmas are parasitic in nature and can attach to specific cells without killing the cells and thus their infection process and progress can go undetected. In some people the attachment of mycoplasmas to the host cell acts like a living thorn; a persistent foreign substance, causing the host's immune defense mechanism to wage war. This allergic type of inflammation often results in heated, swollen, and painful inflamed tissues, like those found in rheumatoid diseases, fibromyalgia and many other autoimmune disorders like lupus and MS, Crohn's and others. In such cases the immune system begins attacking itself and/or seemingly healthy cells. Some species of mycoplasmas also have the unique ability to completely evade the immune system. Once they attach to a host cell in the body, their unique plasma and protein coating can then mimic the cell wall of the host cell and the immune system cannot differentiate the mycoplasma from the body's own host cell.

Mycoplasmas are parasitic in nature because they rely on the nutrients found in host cells including cholesterol, amino acids, fatty acids and even DNA. They especially thrive in cholesterol rich and arginine-rich environments. Mycoplasmas can generally be found in the mucous membrane in the respiratory tract. They need cholesterol for membrane function and growth, and there is an abundance of cholesterol in the bronchial tubes of the respiratory tract. Once attached to a host cell, they then begin competing for nutrients inside the host cells. As nutrients are depleted, then these host cells can begin to malfunction, or even change normal functioning of the cell, causing a chain reaction with other cells (especially within the immune and endocrine systems). Mycoplasmas can even cause RNA and DNA mutation of the host cells and have been linked to certain cancers for this reason. Mycoplasmas can also invade and live inside host cells which evade the immune system, especially white blood cells. Once inside a white blood cell, mycoplasmas can travel throughout the body and even cross the blood/brain barrier, and into the central nervous system and spinal fluid.

**Footnotes**

- Baseman, Joel, et.al., Mycoplasmas: Sophisticated, Reemerging, and Burdened by Their Notoriety, CDC, Journal of Infectious Diseases, Vol 3, No.1, Feb 1997
Dr.Harold Clark, The Intercessor, June 1993, The Road Back Foundation, Delaware OH.

How Mycoplasmas Interact in the Body

To understand how mycoplasmas can cause widespread disease, we must first look at the species' unique properties and interactions with host cells. Unlike viruses and bacteria, mycoplasmas are the smallest free-living and self-duplicating microorganisms, as they don't require living cells to replicate their DNA and growth.

Mycoplasmas are able to hide inside the cells of the host (patient) or to attach to the outside of host cells. Whether they live inside or outside the host cell, they depend on host cells for nutrients such as cholesterol, amino acids, etc. They compete with the host cells for these nutrients which can interfere with host cell function without killing the host cell.

A mycoplasma has very little DNA of its own, but is capable of using DNA from a host cell. When a mycoplasma takes over the DNA of the host cell, anything can happen - including causing that cell to malfunction in many different ways and/or die, or can cause DNA mutation of the host cell. Mycoplasmas attach to host cells with a tiny arm coated in protein which attaches to the protein coating of host cells. For this reason, antibiotics like tetracycline, which are classified as "protein synthesis inhibitors" are often used against mycoplasma infections. While these antibiotics may block this protein attachment and very slowly starve it from the nutrients it needs from host cells to thrive and replicate, it still takes a healthy immune system to actually kill the mycoplasma for good.

Mycoplasmas are highly adaptable to changing environments and can move anywhere in the body, attaching to or invading virtually any type of cell in the body. The mycoplasma adhesion proteins are very similar to human proteins. Once adhered to the host cell, the mycoplasma can completely mimic or copy the protein cell of the host cell. This can cause the immune system to begin attacking the body's own cells; an event that happens in all autoimmune diseases.

Certain Mycoplasma species can either activate or suppress host immune systems, and they may use these activities to evade host immune responses. Mycoplasmas can turn on the chain reaction called an immune system response. This includes the stimulation of pro-inflammatory cytokines (chemical
messengers of the immune system) which is generally found in most autoimmune and inflammatory diseases and disorders. Mycoplasma can also attach to or invade immune system cells, like the very phagocytes (natural killer cells) that are supposed to kill them. Inside these phagocytes, they can be carried to new locations of inflammation or disease - hidden away like a spy who has infiltrated the defending army. When a mycoplasma attaches to a host cell, it generates and releases hydrogen peroxide and superoxide radicals which cause oxidative stress and damage to the surrounding tissues.

The Main Human Mycoplasma Pathogens

<table>
<thead>
<tr>
<th>Pathogen / Implicated Disease (1-6)</th>
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<tbody>
<tr>
<td><strong>Mycoplasma genitalium</strong></td>
</tr>
<tr>
<td>Arthritis, chronic nongonococcal urethritis, chronic pelvic inflammatory disease, other urogenital infections and diseases, infertility, AIDS/HIV</td>
</tr>
<tr>
<td><strong>Mycoplasma fermentans</strong></td>
</tr>
<tr>
<td>Arthritis, Gulf War Syndrome, Fibromyalgia, Chronic Fatigue Syndrome, Lupus, AIDS/HIV, autoimmune diseases, ALS, psoriasis and Scleroderma, Crohn's and IBS, cancer, endocrine disorders, Multiple Sclerosis, diabetes</td>
</tr>
<tr>
<td><strong>Mycoplasma salivarium</strong></td>
</tr>
<tr>
<td>Arthritis, TMJ disorders, Eye and ear disorders and infections, gingivitis, periodontal diseases including even cavities.</td>
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<tr>
<td><strong>Mycoplasma hominis and Ureaplasma urealyticum</strong></td>
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<tr>
<td>Pelvic inflammatory disease, infertility, non-gonococcal urethritis, vaginitis, cervicitis, amnionitis, pyelonephritis, post-partum septicemia, neonatal pneumonia, neonatal conjunctivitis, Reiter's syndrome, peritonitis, wound infections (C-section), low birth weight infants, and premature rupture of membranes.</td>
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<tr>
<td><strong>Mycoplasma pneumonia</strong></td>
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<tr>
<td>Pneumonia, asthma, upper and lower respiratory diseases, heart diseases, leukemia, Steven-Johnson syndrome, polyarthritis or septic arthritis, CNS disorders and diseases, urinary tract infections, Crohn's and Irritable Bowel Syndrome, Guillain-Barr syndrome, polyradiculitis, encephalitis, and septic meningitis, autoimmune diseases.</td>
</tr>
<tr>
<td><strong>Mycoplasma incognitus and Mycoplasma penetrans</strong></td>
</tr>
<tr>
<td>AIDS/HIV, urogenital infections and diseases, Autoimmune disorders and diseases</td>
</tr>
<tr>
<td><strong>Mycoplasma pirum</strong></td>
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<tr>
<td>Urogenital infections and diseases, AIDS/HIV</td>
</tr>
<tr>
<td><strong>Mycoplasma fauicium, M. lipophilum and M. buccale</strong></td>
</tr>
<tr>
<td>Diseases of the gingival crevices and respiratory tract</td>
</tr>
</tbody>
</table>

Murray HW, Masur H, Senterfit LB, Roberts RB. The protean manifestations of Mycoplasma
Baseman, Joel, et.al., Mycoplasmas: Sophisticated, Reemerging, and Burdened by Their Notoriety. CDC, Journal of Infectious Diseases, Vol 3, No.1, Feb 1997
CHRONIC FATIGUE, ALZHEIMER'S, PARKINSON'S & MULTIPLE SCLEROSIS
(Plus other health disorders, including auto-immune disorders)
by: Scott, Donald W., M.Sc.

Donald Scott is a retired high school teacher and university professor who is currently president of the Common Cause Medical Research Foundation and adjunct professor of the Institute of Molecular Medicine. He has extensively researched neurosystemic degenerative diseases over the past five years and has authored many documents on the relationship between degenerative diseases and a pathogenic mycoplasma called Mycoplasma fermentans. His research is based upon solid government evidence. Donald Scott is a veteran of WWII and was awarded the North Atlantic Star, the Burma Star with Clasp, the 1939-1945 Volunteer Service Medal and the Victory Medal.

I - The Mycoplasma a Common Pathogenic Mycoplasma

There are 200 species of mycoplasmas. Most are innocuous and do no harm; only four or five are pathogenic. The Mycoplasma fermentans (incognitus strain) probably comes from the nucleus of the brucellosis bacteria. This disease agent is not a bacteria, and not a virus; it is a mutated form of the brucellosis bacteria, mutated with a visna virus, from which the mycoplasma, is extracted. Dr. Maurice Hilleman, chief virologist for the pharmaceutical company of Merck, Sharp and Dohme, stated that this disease agent is now carried by everybody in North America and possibly most people throughout the world. The mycoplasma used to be very innocuous. Only one person out of 500,000 would get multiple sclerosis; one out of 300,000 would develop Alzheimer's; one out of 1,000,000 would develop Creutzfeldt-Jakob disease. Before the early 1980's, nobody ever died of AIDS because it didn't exist. The mycoplasma is also the disease agent in AIDS, and I have all the documentation to prove it.

BIOWARFARE RESEARCH Between 1942 and the present time, biological warfare research has resulted in a more deadly and infectious form of the mycoplasma. They extracted this mycoplasma from the brucellosis bacteria, weaponized it and actually reduced the disease to a crystalline form. According to Dr. Shyh-Ching Lo, one of America's top, top researchers, this disease agent, the mycoplasma, causes among other things, AIDS, chronic fatigue syndrome, multiple sclerosis, Wegener's disease, Parkinson's disease, Crohn's colitis, Type I diabetes, and collagen-vascular diseases such as rheumatoid arthritis and Alzheimer's. The mycoplasma enters into the individual cells of the body depending upon your genetic predisposition. You may develop neurological diseases if the pathogen destroys certain cells in your brain, or you may develop Crohn's colitis if the pathogen invades and destroys cells in the lower bowel. Once it gets into the cell, it can lie there doing nothing sometimes for 10, 20 or 30 years, but if a trauma occurs like an accident, or a vaccination that doesn't take, the mycoplasma can become triggered. Because it is only the DNA particle of the bacteria, it doesn't have any organelles to process its own nutrients, so it grows by uptaking preformed sterols from its host cell, literally kills the cell, and the cell ruptures and what is left gets dumped into the blood stream.

DOCUMENTED EVIDENCE My conclusions are entirely based upon official documents: 80% are United States or Canadian official government documents, and 20% are articles from peer-reviewed journals, such as the Journal of the American Medical Association, The New England Journal of Medicine, and The Canadian Medical Association Journal. The journal articles and government documents complement each other. We also have a document from Dr. Shyh-Ching Lo which names the mycoplasma as a cause of cancer. Dr. Charles Engel who is with the National Institutes of Health, Bethesda, Maryland, stated at an NIH meeting on February 7, 2000, "I am now of the view that the
probable cause of Chronic Fatigue Syndrome and fibromyalgia is the mycoplasma".

**II - Creation of the Mycoplasma Patent**

Many doctors don't know about this mycoplasma because it was developed by the U.S. military in biological warfare experimentation, and it was not made public. This pathogenic mycoplasma disease agent was patented by the United States military by Dr. Shyh-Ching Lo, who was the top researcher for the military biological warfare research facility. I have the documented patent from the U.S. patent office. A LABORATORY-CREATED PATHOGEN BY THE U.S. MILITARY Researchers in the United States, Canada and Britain were doing biowarfare research with the brucellosis bacteria as well as with a number of other disease agents. From its inception, the biowarfare program was characterized by continuing in-depth review and participation by the most eminent scientists, medical consultants, industrial experts and government officials, and it was top secret. The U.S. Public Health Service also closely followed the progress of biological warfare research and development from the very start of the program, and the Centers for Disease Control (CDC), and the National Institutes of Health (NIH) in the United States were working with the military in weaponizing these diseases. These are diseases which have existed for thousands of years, but they have been weaponized which means they were made more contagious and more effective. And they are spreading. A program developed by the CIA and NIH to develop a deadly lethal pathogen for which humanity had no natural immunity (AIDS) was disguised as a war on cancer and was part of MKNAOMI (ref. Special Virus Cancer Program: Progress Report 8, prepared by National Cancer Institute, Viral Oncology, Etiology Area, July, 1971 and submitted to NIH Annual Report in May, 1971). COMMITTEE ON GOVERNMENT REFORM Many members of the Senate and House of Represent-atives do not know what has been going on. For example, the US Senate Committee on Government Reform had searched the archives in Washington and other places for the document titled The Special Virus Cancer Program: Progress Report No.8 mentioned above and couldn't find it. Somehow they heard I had it, called me and asked me to mail it to them. Imagine. A retired school teacher being called by the United States Senate and asked for one of their secret documents! The United States Senate through their government reform committee is trying to stop this type of government research. BIOLOGICAL WARFARE RESEARCH AGREEMENT All the countries at war were experimenting with biological weapons. In 1942, the governments of the United States, Canada and Great Britain entered into a secret agreement to create two types of biological weapons (one that would kill and one that was disabling) for use in the war against Germany and Japan, who were also developing biological weapons. They primarily focused on brucellosis, and they began to weaponize the brucellosis bacteria.

CRYSRILLINE BRUCELLOSIS In a genuine U.S. Senate Study unclassified on February 24, 1977, the title page of this government record reports that George Merck, of the pharmaceutical company, Merck, Sharp and Dohme (which now makes cures for diseases they at one time created), in 1946, reported to the Secretary of War in the United States that his researchers had produced in isolation for the first time, a crystalline bacterial toxin extracted from brucellosis bacteria. The bacterial toxin could be removed in crystalline form and delivered by other vectors (in nature they are delivered within the bacteria). But the factor that is working in the brucellosis is the mycoplasma. Brucellosis is a disease agent that doesn't kill people; it disables them. But they found that if they had mycoplasma at a certain strength, actually ten to the tenth power, it would develop into AIDS, and the person would die from it within a reasonable period of time because it could bypass our natural human defences. If it was 108, the person would manifest with chronic fatigue syndrome or fibromyalgia. If it was 107, they would present as wasting; they wouldn't die, and they wouldn't be disabled, but they would not be that interested in life, they would waste away (ref. Dr. Donald MacArthur of the Pentagon appearing before a Congressional Committee, June 9, 1969, Department of Defence Appropriations, p.114, 129). Most of us have never heard of brucellosis because it largely disappeared when they began pasteurizing milk, which was the carrier. One salt shaker of this pure disease in a crystalline form could sicken the entire population of Canada. It is absolutely deadly, not in terms of killing the body, but in terms of disabling the body. The advantage of this crystalline disease agent is that it does not show up in blood and tissue tests because the bacteria has disappeared and only the pure disease agent remains. So the doctor thinks that it's all in your head.
CRYSTALLINE BRUCELLOSIS AND MULTIPLE SCLEROSIS About three years ago in Rochester, New York, a gentleman gave me a document and told me, "I was in the U.S. Army, and I was trained in bacteriological warfare. We were handling a bomb filled with brucellosis, only it wasn't brucellosis; it was a brucellosis toxin in crystalline form. We were spraying it on the Chinese and North Koreans." He showed me his certificate listing his training in chemical, biological, and radiological warfare. Then he showed me 16 pages of documents given to him by the U.S. military when he was discharged from the service. It linked brucellosis with multiple sclerosis and stated: "Veterans with multiple sclerosis, a kind of creeping paralysis developing to a degree of 10% or more disability within two years after separation from active service may be presumed to be service-connected for disability compensation. Compensation is payable to eligible veterans whose disabilities are due to service." In other words, "If you become ill with multiple sclerosis, it is because you were handling this brucellosis and we will give you a pension. Don't go raising any fuss about it." The government of the United States, in this official document revealed evidence of the cause of multiple sclerosis, but they didn't make it known to the public, or to your doctor. In a 1958 report, Drs. Kyger and Haden suggest "...the possibility that multiple sclerosis might be a central nervous system manifestation of chronic brucellosis". Testing approximately 113 MS patients, they found that almost 95% also tested positive for brucellosis. We have a document from a medical journal which concludes that one out of 500 people who had brucellosis would develop what they called neurobrucellosis, in other words, brucellosis in the brain which settles in the lateral ventricles where the disease multiple sclerosis is basically located.

CONTAMINATION OF CAMP DETRICK LAB WORKERS A report from the New England Journal of Medicine, 1948, Vol.236, p.741 called "Acute Brucellosis Among Laboratory Workers" shows us how actively dangerous this agent is. The laboratory workers were from Camp Detrick, Frederick, Maryland where they were developing biological weapons. Even though these laboratory workers had been vaccinated, wore rubberized suits and masks, and worked through holes in the compartment, many of them came down with this awful disease because it is so absolutely and terrifyingly infectious. The article was written by Lt. Calderone Howell, Marine Corps, Captain Edward Miller, Marine Corps, Lt. Emily Kelly, United States Naval Reserve and Captain Henry Bookman. They were all military personnel engaged in making the disease agent brucellosis into a more effective biological weapon.

III - Covert Testing of the Mycoplasma Testing Brucellis upon an Unsuspecting Public

Documented evidence proves that the biological weapons they were developing were tested on the public in various communities without their knowledge or consent. The government knew that crystalline brucellosis would cause disease in humans. Now they needed to determine how it spread, and the best way to disperse it. They tested dispersal methods for Brucella suis and Brucella melitensis at Dugway Proving Ground, Utah, June and September 1952. Probably, 100% of us now are infected with Brucella suis and Brucella melitensis. (ref. p.135, table 4 of Special Virus Cancer Program: Progress Report 8) . Another government document recommended the genesis of open air vulnerability tests, and covert research and development programs to be conducted by the army and supported by the Central Intelligence Agency. At that time, the government of Canada was asked by the government of the United States to cooperate in testing weaponized brucellosis, and Canada cooperated fully with the government of the United States. They wanted to determine (i) if mosquitoes will carry the disease and (ii) if the air will carry it. A government report stated that "...open air testing of infectious biological agents is considered essential to an ultimate understanding of biological warfare potentialities because of the many unknown factors affecting the degradation of micro-organisms in the atmosphere".

TESTING BRUCELLOSES VIA MOSQUITO VECTOR IN PUNTA GORDA

A report from The New England Journal of Medicine, August 22, 1957, p.362 reveals that one of the first outbreaks of chronic fatigue syndrome was in Punta Gorda, Florida, back in 1957. It was a strange coincidence that a week before these people came down with chronic fatigue syndrome, there was a huge influx of mosquitoes. The National Institutes of Health claimed that the mosquitoes came from a forest fire 30 miles away. When the forest fire broke out, the mosquitoes all said, "Well, let's go over to Punta Gorda - there will be a bunch of people over there, we can have a picnic, and then
we will go home'. The truth is that those mosquitoes were infected in Canada by Dr. J.B. Reed at Queen's University. They were bred in Belleville, Ontario, and taken down and released in Punta Gorda. Within a week, the first five cases ever of chronic fatigue syndrome were reported to the local clinic in Punta Gorda, and it continued until finally 450 people were ill with the disease.

**TESTING BRUCELLOSIS VIA MOSQUITO VECTOR IN ONTARIO**

The government of Canada established the Dominion Parasite Laboratory in Belleville, Ontario, and raised 100 million mosquitoes a month which were shipped to Queen's University and certain other facilities to be infected with this disease agent. The mosquitoes were then let loose in certain communities in the middle of the night so they could determine how many people would become ill with chronic fatigue syndrome, or fibromyalgia, which was the first disease to show. One of the communities they tested it on was the St. Lawrence Seaway valley all the way from Kingston to Cornwall in 1984. They let out absolutely hundreds of millions of infected mosquitoes. Over 700 people in the next four or five weeks developed myalgic encephalomyelitis, or chronic fatigue syndrome.

**IV - Other Secret Government Testing Mad Cow Disease in the Fore Indian Tribe**

At the infamous Japanese Camp 731 in Manchuria, they contaminated prisoners of war with certain disease agents. They also established a research camp in New Guinea in 1942, and experimented upon the Fore Indian tribe, and inoculated them with a minced-up version of the brains of diseased sheep containing the visna virus which causes mad cow disease (Creutzfeldt-Jakob disease which is known to you as mad cow disease, but which was known to the Fore Indian tribe as kuru). About five or six years later, after the Japanese had been driven out, the poor people of the Fore tribe developed what they called kuru which was their word for wasting, and they began to shake, lose their appetites, and die. The autopsies revealed that their brains had literally turned to mush. They had contracted mad cow disease from the Japanese experiments. When World War II ended, the Japanese General Doctor who was in charge of biological warfare experimentations in Japan, Dr. Ishii Shiro, was captured. They gave him the choice of a job with the United States army or execution as a war criminal. Not surprisingly, Dr. Ishii Shiro chose to work with the United States military to demonstrate how they had created mad cow disease in the Fore Indian tribe. In 1957, when the disease was beginning to blossom in full among these Fore Indian people, Dr. Carleton Gajdusek of the National Institutes of Health of the U.S. headed down to New Guinea to determine how the minced-up brains of the visna-infected sheep affected these people. He spent a couple of years in New Guinea studying the Fore tribe, wrote an extensive report on it, and won the Nobel Prize for "discovering" kuru disease (also known as mad cow or Creutzfeldt-Jakob disease) in the Fore Indian tribe in New Guinea.

**TESTING CARCINOGENS IN RUSSIA**

In 1953, the Americans developed a carcinogenic chemical which they wanted to test, but they didn't want to test it in the United States so they flew over Russia, accidentally wandered off course, and sprayed this stuff. Many people started getting cancer. And the U.S. had some jokes about this. One American researcher, Dr. Maurice Hilleman of Merck, Sharp and Dohme, joked, "We are going to win the next Olympics because all the Russians are going to turn up with 40-pound tumours." They thought it was a big joke.

**TESTING CARCINOGENS IN WINNIPEG**

Next they said, "How about testing it in Canada?" In 1953, the U.S. asked the government of Canada if they could test this carcinogenic chemical over the city of Winnipeg. It was a big city with 500,000 people, miles from anywhere. They sprayed the chemical in a 1,000% attenuated form, which they said would be so watered down that nobody would get very sick. However, if people came to clinics with a sniffle, a sore throat, or ringing in their ears, the researchers would be able to determine what percentage would have developed cancer if it had been full strength. When we located evidence that the Americans had tested this carcinogenic chemical over the city of Winnipeg in 1953, and informed the government that we had this evidence, they denied it. However, finally, on May 15, 1997, a story out of the Canadian Press in Washington, D.C. by Robert Russo, published in the Toronto Star, stated that the Pentagon of the United States admitted that in 1953 they had obtained permission from the government of Canada to fly over the city of Winnipeg and spray this crap out, and it sifted down on kids going to school, housewives hanging out their laundry, and people going to work. US Army planes and trucks released the chemical 36 times between July and August 1953. The chemical used was zinc...
cadmium sulfide, a carcinogen. They got their statistics, which indicated that if it had been full strength, approximately a third of the population of Winnipeg would have developed cancers over the next five years. The Pentagon called a press conference to admit what they had done. One professor, Dr. Hugh Fudenberg, MD, who was nominated twice for the Nobel Prize wrote a magazine article which stated that the Pentagon has come clean on this because two researchers up in Sudbury, Ontario, Don Scott and his son Bill Scott had been revealing this to the public. The US Army actually conducted a whole series of simulated germ warfare tests in Winnipeg. The Pentagon lied about the tests to the mayor, saying that they were testing a chemical fog over the city, which would protect Winnipeg in the event of a nuclear attack. A report commissioned by US Congress, chaired by Dr. Rogene Henderson, lists 32 American towns and cities used as test sites as well.

V - Brucellosis Mycoplasma & Disease

AIDS The AIDS pathogen was created out of a brucellosis bacteria mutated with a visna virus; then the toxin was removed as a DNA particle called a mycoplasma. They used the same mycoplasma to develop disabling diseases like MS, Crohn's colitis, Lyme disease etc. In a United States congressional document of a meeting held June 9, 1969, the Pentagon delivered a report to Congress about biological weapons (described on page 129 of the document). The Pentagon stated, "We are continuing to develop disabling weapons." Dr. MacArthur, who was in charge of the research said, "We are developing a new lethal weapon, a synthetic biological agent that does not naturally exist, and for which no natural immunity could have been acquired." Think about it. If you have a deficiency of acquired immunity, you have an acquired immunity deficiency. Plain as that. AIDS. In laboratories throughout the United States and a certain number in Canada, including the University of Alberta, the U.S. government provided the leadership for the development of the AIDS virus for the purpose of population control. After they had it perfected, they sent medical teams from the Centers for Disease Control to Africa and other mid-eastern countries where they thought the population was becoming too large. They gave them all a free vaccination for smallpox. Five years after receiving this smallpox vaccination, 60% of them were suffering from AIDS. They tried to blame it on a monkey, which is nonsense. There was a report in the newspapers a while back about a professor at the University of Arkansas who claimed that while studying the tissues of a dead chimpanzee, she found the HIV virus. The chimpanzee that she had tested was born in the United States 23 years earlier. It had lived its entire life in a U.S. military laboratory where it was used as an experimental animal for the development of these diseases. When it died, its body was shipped to a storage place where it was deep-frozen and stored in case they wanted to analyze it later. Then they decided that they didn't have enough space for it, so they said, "Anybody want this dead chimpanzee?" and this researcher from Arkansas said, "Yes. Send it down to the University of Arkansas. We are happy to get anything that we can get." They shipped it down and she found the HIV virus in it. That virus was acquired by that chimpanzee in the laboratories where it was tested.

CHRONIC FATIGUE Chronic fatigue syndrome is more accurately called myalgic encephalomyelitis, not chronic fatigue syndrome. That nomenclature was given by the National Institutes of Health in the United States because they wanted to downgrade and belittle the disease. An MRI of the brain of a teenage girl who had chronic fatigue syndrome displayed a great many scars or punctate lesions in the left frontal lobe area where portions of the brain had literally dissolved and had been replaced by scar tissue. This caused cognitive impairment, memory impairment, etc. And what was the cause of the scars? The mycoplasma. So there is very concrete physical evidence of these tragic diseases even though doctors continue to say they don't know where it comes from or what they can do about it

APPEALS TO CANADA PENSION

Many people with chronic fatigue syndrome, myalgic encephalo-myelitis and fibromyalgia who apply to the Canada Pension Plan will be turned down because they cannot prove that they are ill. Over the past year I have conducted several appeals to Canada Pension and Workers Compensation on behalf of people who have been turned down. I provided documented evidence of these illnesses, and they were all granted their pensions on the basis of the evidence that I provided. In March of last year, for example, I appealed to the Workers' Compensation on behalf of a lady with fibromyalgia who had been denied her pension back in 1993. The vice-chairman of the board came up to Sudbury to hear the appeal, and I showed him a number of documents which proved that this lady was physically ill
with fibromyalgia. It was a disease which caused physical damage, and the disease agent was a mycoplasma. The guy listened for three hours and then he said to me, "Mr. Scott, how is it I have never heard of any of this before? I said, "We brought a top authority in this area into Sudbury to speak on this subject and not a single solitary doctor came to that presentation."

**VI - Testing for the Presence of Mycoplasma in your Body - the Polymerase Chain Reaction Test**

Information is not generally available about this agent, because first of all, the mycoplasma is such an infinitely small disease agent. A hundred years ago certain medical theoreticians conceived that there must be something smaller than the bacteria and the virus, which are the most common living forms of disease agents. This pathogenic organism is so infinitely small that normal blood and tissue tests will not reveal the source of the disease. Your doctor may diagnose you with Alzheimer's and he will say, "Golly, we don't know where Alzheimer's comes from. All we know is that your brain begins to deteriorate, cells rupture, the myelin sheath around the nerves dissolves, and so on." Or if you have chronic fatigue syndrome, the doctor will not be able to find any cause for your illness with ordinary blood and tissue tests. This mycoplasma couldn't be detected until about 30 years ago when they developed the polymerase chain reaction test in which they examine a sample of your blood, remove damaged particles, and subject that damaged particle to a polymerase chain reaction. This causes the DNA in the particle to break down. Then they place it in a nutrient which causes the DNA to grow back into its original form. If they get enough of it they can recognize what it is, and determine whether brucellosis or another kind of agent is behind that particular mycoplasma.

**THE BLOOD TEST**

If anybody in your family has myalgic encephalomyelitis, fibromyalgia, multiple sclerosis, or Alzheimer's, you can send a blood test to Dr. Les Simpson in New Zealand. If you are ill with these diseases, your red blood cells will not be normal donut-shaped blood cells capable of being compressed and squeezed through the capillaries, but will swell up like cherry-filled donuts, which cannot be compressed. The blood cells become enlarged and distended because the only way the mycoplasma can exist is by uptaking preformed sterols from the host cell. One of the best sources of preformed sterols is cholesterol, and cholesterol is what gives your blood cells flexibility. If the cholesterol is taken out by the mycoplasma, the red blood cell swells up, doesn't go through and the person begins to feel all the aches and pains, and all the damage it causes to the brain, the heart, the stomach, the feet and the whole body because blood and oxygen is cut off. And that is why people with fibromyalgia and chronic fatigue syndrome have such a terrible time. When the blood is cut off from the brain, punctate lesions appear, because those parts of the brain die. It will get into portions of the heart muscle, especially the left ventricle, and those cells will die. Certain people have cells in the lateral ventricles of the brain that have a genetic predisposition to admit the mycoplasma, and it causes the lateral ventricles to deteriorate and die and this leads to multiple sclerosis which will progress until they are totally disabled and frequently die prematurely. It will get into the lower bowel and parts of the lower bowel will die and cause colitis. All of these diseases are caused by the degenerating properties of the mycoplasma.

About two months ago a gentleman in Sudbury phoned me and told me he had fibromyalgia. He applied for Canada Pension and was turned down because his doctor said it was all in his head and there was no external evidence. I gave him the proper form and a vial, and he sent his blood to Dr. Les Simpson of New Zealand to be tested. He did this with his family doctor's approval, and the results from Dr. Simpson showed that only 4% of his red blood cells were functioning normally and carrying the appropriate amount of oxygen to his poor body, whereas 83% were distended, enlarged and hardened, and wouldn't go through the capillaries without an awful lot of pressure and trouble. This is the physical evidence of the damage that is done.

**THE ECG TEST**

You can also ask your doctor to give you a 24-hour Holter ECG. You know, of course, that an electrocardiogram is a measure of your heart beat, which shows what is going on in the right ventricle, the left ventricle, and so on. Tests show that 100% of patients with chronic fatigue syndrome and fibromyalgia have an irregular heart beat. At various periods of time, during the 24
hours, the heart, instead of working happily away, going "bump-BUMP, bump-BUMP", every now and again, it will go "buhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuhbuh
Other recommended reading is Osler's Web by Hillary Johnson and Emerging Viruses: Aids and Ebola by Leonard Horowitz. Don Scott also produces The Journal of Degenerative Diseases.

You may contact Donald Scott at: 190 Mountain St., Ste. 405, Sudbury, Ontario, Canada P3B 4G2. 705-670-0180. Note: Dr. David Webster at Sudbury General Hospital, a wonderful person, with whom I have had conversations about these awful diseases can tell your doctor about the Blood Volume test.

**Colloidal Silver may be the answer. By John Claydon D.Hom**

M.S. patients, taking colloidal silver (typically one pint a day) have made excellent improvements in their symptoms. One such person, Nadine A Wooley, had brain scans taken before and after one years use of the colloidal silver. Before, showed typical white areas in the brain showing typical M.S. lesions. A year after taking one pint of silver daily a subsequent scan showed no lesions.

It may be that mycoplasma can be eliminated with colloidal silver. We also recommend the use of a product called Zell-Oxygen. This is a live cell very small, nutritional yeast (helps fight pathogenic fungi in the body including Candida Albicans, and is extremely well tolerated by persons who have allergic reactions to other forms of yeast (usually cooked or sterilised).

The Zell yeast provides enzymes that repair the structure and function of the mitochondria of every cell. The DNA of cellular mitochondria are damaged very easily by virus, bacteria, mycoplasma, toxins etc. One of the roles of the mitochondria is to act as a ‘biological spark plug’ to get all the cells processing nutrients and, especially, oxygen. Most of the energy required for day to day functioning, including a proper activity of the immune system rests upon sufficient cellular metabolism. It is for these reasons that the administration of Zell-Oxygen proves so effective in improving the immune system, aids detoxification, and helps improve many chronic health disorders especially M.E. I also recommend a protein digesting enzyme that targets 'dead' cells and scars, called Serrapeptase. M.S. sufferers are finding that this helps to promote healing of scar tissue internally and promote a healing process. This has the effect of helping to improve mobility. Serrapeptase has a wide role clinically and is perfectly safe to take long term. It may also help regenerate any tissue ravaged by mycoplasma.

It is also interesting to note that the damaged red blood cells referred to earlier in the above mycoplasma article, can be corrected with the use of Zell Oxygen. Until we know more, I will be encouraging the use of Zell-Oxygen, and colloidal silver, and Serrapeptase for all the diseases mentioned in the above mycoplasma article.

Excerpt from the book: HOW TO BEAT MULTIPLE SCLEROSIS by Nadine A Wooley.

Colloidal Silver is a suspension of extremely fine sub- microscopic particles (0.15-.001 microns) of pure silver suspended in water by a positive electric charge on each particle. A powerful germicidal, silver is an exceptional metal in that it is non-toxic to the human body, but lethal to over 650 disease-causing bacteria, viruses, fungi, parasites, and moulds; while conventional pharmaceutical antibiotics are typically effective against only 6 or 7 types of bacteria. Some new strains of bacteria classified as MDR (Multiple Drug Resistant) have proven to be resistant to ALL pharmaceutical antibiotics, but NOT to Colloidal Silver due to different germicidal mechanisms of deactivation.

The function of our immune system is to respond to invading micro organisms by producing and or sensitizing lymphocytes that will recognize and destroy the invaders. Autoimmune disorders occur when these reactions unexplainably start fighting our body's own cells and tissues, producing a variety of disorders, like MS.

Many scientists believe that the MS immune response is triggered by a virus. Recently, certain bacteria also have been associated with MS. According to the scientific research, there is no bacteria, fungus or virus that silver does not kill. Consequently, it seems very likely it will kill the MS “trigger.” As Sais points out: Regular ingestion of Colloidal Silver can act as a second immune system by assisting the body in the war against invading micro-organisms. Unlike pharmaceutical antibiotics,
which destroy beneficial enzymes. Colloidal Silver leaves these tissue cell enzymes intact. Colloidal Silver, if used sensibly, is completely non-toxic and will not harm the immune system in any way. The Environmental Protection Agency's Poison Control Centre reports no toxicity listing for Colloidal Silver.

In testing by the UCLA Medical Research Centre, silver has not been found to interact with any of more than 400 drugs. This is significant for MS patients, who are often taking a number of drugs. I began drinking purified water fortified with Colloidal Silver on March 12, 1999, and I have not felt this good in ten years. My neurologist, Dr. Grimm, is amazed and dumbfounded. He commented that "Never has a chronic/progressive patient walked into my office and announced that she will be walking soon without a cane. Never has a chronic patient of mine anticipated not having another exacerbation."

When I began drinking the Colloidal Silver on March 12, I could not walk a city block without holding someone's arm with one hand and a cane with my other hand. In fact, unassisted, I could only walk between my kitchen counters. The first week I began drinking Colloidal Silver, I quit using the walker in my house. The second week I walked 4/10ths of a mile up my street holding Les' arm and without a cane. I have continued to increase the distance I can walk. On the graph of improvement, I continue to go up.

**How does silver work? (According to Sais:)**

There are three mechanisms of deactivation that silver utilizes to incapacitate disease-causing organisms. They are: Catalytic Oxidation; Reaction with Cell Membranes; and Binding with the DNA of disease organisms to prevent unwinding. Catalytic Oxidation: in its atomic state, silver has the capacity to absorb oxygen and act as a catalyst to bring about oxidation. Atomic (nascent) oxygen absorbed onto the surface of silver ions in solution will readily react with the sulfhydryl (-S-H) groups surrounding the surface of bacteria or viruses to remove the hydrogen atoms (as water), causing the sulfur atoms to form an R-S-S-R bond, blocking respiration and causing the bacteria to expire. Employing a simple catalytic reduction/oxidation reaction, Colloidal Silver will react with any negative charge presented by the organism's transport or membrane proteins and deactivate them.

Reaction with Bacterial Cell Membrane: there is evidence that silver ions attach to membrane surface radicals of bacteria, impairing their cell respiration and blocking their energy transfer system. One explanation is based on the nature of enzyme construction: specific enzymes are required for a given biochemical activity to take place. Enzyme molecules usually require a specific metallic atom as part of the molecular matrix in order to function. A metal of lower valance in the enzyme complex will prevent the enzyme from functioning normally. Silver, with a valance of plus 2, can replace many metals with a lower or equal valance. Binding with DNA: studies with Pseudomonas aeruginosa, a tenacious bacteria that is difficult to treat, demonstrated that as much as 12 percent of silver is taken up by the organism's DNA without destroying the hydrogen bonds holding the lattice together but this nevertheless prevents the DNA from unwinding, an essential step for cellular replication to occur.

Silver is not new to MS. Its antibiotic qualities were first investigated in regards to MS during the 1930s. At that time, spirochetal bacteria were observed in Syphilis and MS patients. Silver kills the bacteria and was used topically in a salve to treat Syphilis. Research on MS in conjunction with silver then began. Following Penicillin's use as a Syphilis treatment in the 1940s, MS research with silver was unfortunately halted because of silver's great expense. The original research was never concluded, and no further research occurred for decades. In the last several decades, the process of creating Colloidal Silver has been developed and refined. During the manufacturing process, electricity passes through the purified water as the silver particles are being introduced and causes the silver to break into a microscopic "dust" Lightning actually passes through the water. My nickname for it is "liquid lightning." The result is water with electrically charged, antiviral, antibacterial, and antifungal properties.

Sais explains that Colloidal Silver may be taken orally or topically (applied directly to the skin). It can be taken vaginally or anally, atomized or inhaled into the nose or lungs or dropped into the eyes. Liquid silver, as well as new gel formations, may be applied directly to the skin. A few drops on a Q-tip
or Band-Aid may be used to disinfect any wound or sore. Liquid silver can also be injected. Colloidal Silver does not affect any of the body’s friendly bacteria.

It does not have any negative effects on the natural flora in the bowels. It kills disease-causing organisms very quickly upon contact. It is both a remedy for illness and a preventative treatment. So why haven't we heard about this before? One reason is that although we have known about silver's medical properties for some time, the process of manufacturing a good quality, drinkable silver water was not developed until a few decades ago. Another reason may be that Colloidal Silver does not offer the profit possibilities major American companies are seeking. Silver cannot be patented. The manufacturing process for Colloidal Silver cannot be patented. I drink one 16 ounce bottle per day.

Dr. Grimm has warned me to wait until the results are in from a scientific study regarding silver and specifically MS. I can't. You have to know about this. As I write this, I have been using the silver solution for just under three months and I can walk further than I have in seven years. This is too exciting. Study results will come in over the course of the next two years. However, that is too long to wait. It is my recommendation that MS patients begin using Colloidal Silver on a daily basis. As noted, I drink one bottle, 16 ounces per day. That may be excessive, but it has worked for me. Do not use less than 4 ounces per day. A maintenance program is figured at 1 ounce per 100 pounds of body weight. But you are not looking for maintenance.

You want to boost your immune system so it can fight the invading virus or bacteria. Colloidal Silver works in your body like a second immune system, and that is what we MS patients need! And remember, we are not waiting for FDA approval; this stuff is sold "over-the-counter" Try the amount I take. (Remember, your body will pass what it does not use. Colloidal Silver is not addictive, and you will not build up a resistance.) What have you got to lose? A few dollars? Health is wealth. When test results come in positive for Colloidal Silver helping MS patients, we can only hope that our insurance agencies will encourage its use and decide to pick up the cost.

Taken from ‘HOW TO BEAT MULTIPLE SCLEROSIS (and the things your doctors didn’t tell you)’ By Nadine A Wooley, Self published, MS UPBEAT, Portland, Oregon. USA. c2000

**Colloidal silver cured M.S. By Nancy Delise**

What follows is the story of Nancy Delise, who, over the years, has utilized retail colloidal silver, colloidal silver made with a basic generator, basic colloidal silver enhanced with H2O2 (orally), and finally IV Silver (Argentyn 23 by Natural-Immunogenics - Available only to MD's).

**Condition: MS Before silver**

I have been on Betaseron since it came on the market—for 6 or 7 years? I would say it did as promised; I have had no exacerbation since I began the injections. However, everyday I hate to get up to see what additional symptom I have to add to my list to get used to.

My right had is numb, my feet, especially my toes are numb. When I get hot or tired my right leg does not lift well. It drags when I walk. After a day at work, I practically have to crawl to my car. I must hold on to a wall at all times. I really should use a cane. I cannot even go up a curb without holding on to someone or something. No way can I climb a ladder. When I sit for any length of time, my legs stiffen and get spasms and I have to wait awhile before I can walk. It appears that I have had too much to drink. I really should use a cane, but usually I can take my companion’s arm to get to my car.

If I sit on the floor for any reason, like play with my grandchildren, I must first get on my knees, then on all four’s, then finally I can get up. Just like a cow. I cannot use help getting up from the floor, I need more control. I sit on the floor as little as possible. When it is hot, I must wear a cold pack vest or I cannot walk. My feet are hot all the time, and I cannot sleep unless my feet are uncovered. I have night paralysis. I must throw my body in order to turn to another side. My legs are locked in the foetal position and it is a real chore to get them unlocked and able to walk. I must use a cane to get to the bathroom during the night. It is about ten feet from my bed.
Jane Wyman has become my good friend with the Poise pads. I cannot go out without the Ultra Poise pads. If I know I will be away from a bathroom for any length of time, I must use Depends. It goes without saying, I must use the pads at night, also.

**Colloidal silver oral use**

I drank 2 oz of Silver water twice a day - in the morning & at three PM. Day four I begin to drink 8 oz of Silver water two times per day. I seem to have more energy and the end of the day seems to come a little later. I do not drag as much to my car.

Day 12 The night paralysis seems to be easing. I can get out of bed with more ease Day 14 thru Day 18 My fingers and toes are tingling more and more. My toes are aching. As the days go by my fingertips seem to be aching, also. Day 20 I seem to have surreal feelings in my fingers. It’s like a far away out of body feeling. They still ache.

Day 21, I am getting out of bed much easier and quicker. I climbed a ladder at work, and I am not nearly so tired when I leave work. I can actually walk to my car without holding on to the wall. I did some things on the floor at work, and was able to get up without too much trouble.

Week four, the bottom of my feet are tingling, and I could feel whiskers on Mike’s face (a surreal feeling). I could feel cool bathroom tile on bottom of my feet. My legs ached all night. It was very painful, I wanted to scream out. My legs hurt a great deal. The next morning I was able to walk further than I had in years. Mike and I walked about four blocks that morning. I feel stronger and stronger every day.

Week five, more and more feeling in both fingers and toes every day, less surreal and more natural. Both toes and finger get cold.

Week 10, Seem to have small changes every day. Again my toes ached for several days, then I had more feeling in my toes. It’s as though I have a non feeling pad at the bottom of my feet, but feeling all the way around. Like an animal’s paw with the padded bottom. It seems I hurt for a few days, then something feels better.

Week 12, I feel like a caterpillar in a cocoon. I wonder if they have pain during the metamorphosis. The bottom of my feet are no longer numb, the fingers on my right hand tingle only at the very tips. I don’t even think about lifting a heavy container with my right hand. For years, I wouldn’t dare lift, or I would drop whatever I was holding. I poured coffee from a pot without even thinking about it, until I noticed myself. Doing it. There is NO WAY I could be working the hours I have this Christmas, if not for the water. Last year, I had to wear my cooling vest all day every day, and when I went home I could barely walk to my car. Some days I literally dragged my right leg to get to my car. I had to hold on to the building to get around the corner and into my car. When I got home I actually crawled on my hands and knees to get up the steps. This year I never once had to wear my cooling vest. I walk normally to my car at the end of the day, and the steps are not too much of a problem. I still go up one leg only, but it is stronger. The fatigue is minimized, also. I’ve worked many more hours this year than last.

Week 14: I started making my own water about three weeks ago, and I’ve had to send samples to San Antonio for testing. It seems the probe they sent me was not working to full potential, and for about a week I was drinking water with very minimal amounts of silver. After the week I KNEW IT!!! I was regressing. Things were not working so well, again. I was regressing. Thankfully we figured out the problem and within a couple of days I was back on track. Thank God. This set back has convinced me even more. As if that were possible. I have my life back. I will never give up silver water. Week 20: Christmas Week. I had 16 people for dinner Christmas eve. I had 7 people for dinner Christmas day, I worked 11 hours the day after Christmas, and I had 14 people for dinner the next day. That is four days out of four I entertained at my house. I can’t remember when I did something like that. I still have night paralysis, but not nearly as bad as it used to be, and I have a lot of stiffness still when I sit
a long time, but nothing near as bad as it used to be. My energy level is very high.

August 2002
My MS Update This is the second anniversary of my long, but wonderful journey with colloidal silver (CS). I am a 59-year-old female who had relapsing remitting MS for 31 years. About 1995 it changed to secondary progressive MS. Thus began my long road of decline. Everyday I got worse. When I discovered CS I could barely walk. I was beginning to use a cane. I could not even go up on the curb without aid. My prognosis was grim. I had some knowledge of the great properties of silver, so the idea of CS intrigued me. I researched CS. What did I have to loose?

I began drinking 16 oz per day. In about three weeks I began to notice a difference. You already have a log of my first year’s progress. I seemed to reach a plateau about this time. I did not improve, BUT I never got worse.

I have since had an MRI and it showed that at this time Aug 2001, I no longer had MS. I have had no new lesions for well over a year. What I was working on at the time is to now repair the damage. Since the damage is to the myelin and not the central nervous system, I was quite confident I could improve.

1 year- 6 months Hydrogen Peroxide Added.
I have researched adding hydrogen peroxide to the CS. One drop of H2O2 per 2 oz. of CS. I learned this would cause the tiny silver particles to break up into even more minute particles. After 15 minutes, the peroxide was evaporated out of the CS, so it is not harmful to the body, but the tinier particles of silver got into the blood stream quicker. All this time it was a slow process because by the time the silver got to the myelin where it was needed, it was so diluted, it couldn’t penetrate the lesions and kill the mycoplasma (MS virus). Within a week I began to feel old symptoms again. This is what I call a healing crisis: I would get symptoms of the MS as the virus was dying and the dying pathogen aggravated the nerves, so for 2-4 days I would feel like I was having varying degrees of exacerbation. After a short period, it would end and I was improved again. If I had known about this earlier, I am convinced my recovery time would have decreased a great deal.

1 year-9 months: I am sure there is a way to go even quicker......... I began to research IV drips. There are cases of HIV-AIDS infected patients going into complete remission after three infusions. I worked on this project for about six weeks. I finally found someone with a protocol of infusing CS intravenously. I also found a doctor willing to work with me and give this a try.

1 year-11 months: Colloidal Silver IV Administered
First IV: I had my first IV. By that evening I had my first healing crisis; my legs became extremely heavy (like they were 2 years ago). My fingers tips were still numb, but the numbness was extremely exaggerated. All was better at day four.

Second IV a week later: My legs are again aching a great deal, the numbness in my fingers is very intense. It almost feels like they are not attached to me. All better by day three.

Third, fourth, fifth IV: Each time I experienced a reverse of some symptoms I had either forgotten about over the last 40 years, or didn’t realize over the years were actually MS symptoms. I’ve practically no problems at all. I feel better then I have in 15 years. I will have no more IV’s, but I will NEVER stop drinking CS.

If I had known about the IV’s I probably would have had full recovery even sooner. I am quite sure the old lesions are going away. I am anxious for another MRI to prove this also.

TWO YEAR ANNIVERSARY: No more MS, no more symptoms. Most myelin repaired.

PS: My friend, also an MS patient is on the IV drip. She also no longer has MS (By her MRI), but she was worse than me, and not able to get out of her wheel chair. Since IV’s she has given up all her spasm medication and has begun to take STEPS ON HER OWN.
I would be happy to share what I've learned with anyone. Call me, Nancy Delise, @ 708-442-6229

Disclaimer: Regenerative Nutrition advocates a holistic approach to natural health and wellbeing. The body's ability and power to heal depends upon the totality of diet, nutrition, lifestyle and environmental factors. No claims for the cure of any disease is intended, or implied. Always consult a health care practitioner when combating disease states. The statements in this article have not been approved by the FDA.
United States Patent [19]
Lo

[54] PATHOGENIC MYCOPLASMA
[75] Inventor: Shyh-Ching Lo, Potomac, Md.
[73] Assignee: American Registry of Pathology, Washington, D.C.
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[56] References Cited
PUBLICATIONS

Primary Examiner—Christine M. Nucker
Assistant Examiner—D. R. Preston
Attorney, Agent, or Firm—Venable, Baetjer, Howard & Civiletti

ABSTRACT
The invention relates to a novel pathogenic mycoplasma isolated from patients with Acquired Immune Deficiency Syndrome (AIDS) and its use in detecting antibodies in sera of AIDS patients, patients with AIDS-related complex (ARC) or patients dying of diseases and symptoms resembling AIDS diseases. The invention further relates to specific DNA sequences, antibodies against the pathogenic mycoplasma, and their use in detecting DNA or antigens of the pathogenic mycoplasma or other genetically and serologically closely related mycoplasmas in infected tissue of patients with AIDS or ARC or patients dying of symptoms resembling AIDS diseases. The invention still further relates to a variety of different forms of vaccine against mycoplasma infection in humans and/or animals.

2 Claims, 39 Drawing Sheets
PATHOGENIC MYCOPLASMA

The invention described herein was made in the course of work under a grant or award from the United States Department of the Army.

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S. patent application Ser. No. 265,920, filed Nov. 2, 1988, now abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 875,535, filed Jun. 18, 1986, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a novel strain of mycoplasma isolated from a patient with AIDS. The mycoplasma is closely related to a species of human mycoplasma, M. fermentans. Upon characterization of this mycoplasma, it may be classified as a unique strain within the species M. fermentans incognitus. This novel strain of mycoplasma is referred to hereinafter as the incognitus strain or M. fermentans incognitus.

The invention also relates to the use of the mycoplasma M. fermentans incognitus as well as all strains of M. fermentans in detecting specific antibodies in sera of patients with AIDS or an acute fulminant systemic disease and/or animals and its use as a vaccine against infection by the mycoplasma. The invention further relates to incognitus strain-specific antibodies and cross-reactive which later break up into individual cells that are capable of passing through membrane filters of pore size 0.45 µm or even 0.22 µm.

A trilaminar cytoplasmic membrane contains sterols, phospholipid and proteins. Therefore, the cells are generally susceptible to polyene antibiotics and to lysis by digitonin.

Replication of the Mycoplasma genome may precede cytoplasmic division resulting in multinucleate filaments before individual cells are delimited by constriction. Budding can also occur.

Most Mycoplasma species are facultatively anaerobic, and all known species are chemoorganotrophic. The fermentative species of Mycoplasma utilize sugars such as glucose, while non-fermentative species can utilize arginine.

Known mycoplasmas may be grown on complex media, such as Hayflick medium, while fastidious mycoplasmas may be grown on diphasic SP-4 medium. The colonies are usually of the "fried egg" type, i.e., an opaque, granular central region, embedded in the agar, surrounded by non-granular surface growth. The optimal growth temperature of mammalian strains is 36°-37° C.

Many species of Mycoplasma produce weak or clear haemolysis which appears to be due to the secretion of H₂O₂. This H₂O₂ secretion is believed to be responsible for some aspects of the mycoplasmas' pathogenicity. Known mycoplasmas are commonly sensitive to chloramphenicol and tetracyclines.

The Mycoplasma genus currently consists of more than 60 known species which are differentiated on the basis of various tests, including utilization of glucose and mannose, arginine hydrolysis, phosphatase production, the "film and spots" reaction and haemadsorption. M. fermentans antibodies (i.e. antibodies to homologous antigenic determinants), including monoclonal antibodies of each, which are useful in detecting incognitus strain antigens in infected tissue of patients or animals. The invention also relates to incognitus strain-specific DNA probes which are useful in detecting incognitus strain genetic materials in infected tissues of patients or animals. Incognitus strain genetic materials may also be detected in infected humans or animals by using specific incognitus strain DNA sequences and the polymerase chain reaction ("PCR") (U.S. Pat. No. 4,683,202 incorporated herein by reference).

The ability to monitor AIDS or other acute fulminant systemic disease status can be of great value. In addition to improving prognostication, knowledge of the disease status allows the attending physician to select the most appropriate therapy for the individual patient, e.g., highly aggressive or less aggressive therapy regimens. Because of patient distress caused by more aggressive therapy regimens, it is desirable to distinguish those patients requiring such therapies. It has been found that M. fermentans incognitus is more directly associated and functional deficits of the infected organ systems and is capable of distinguishing such patients.

Mycoplasma is a genus of cell wall-less sterol-requiring, catalase-negative pathogens commonly found in the respiratory and urogenital tracts of man and other animals. The cells of Mycoplasma are typically non-motile and pleomorphic, ranging from spherical, ovoid or pear-shaped to branched filamentous forms. Filaments are the typical forms in young cultures under optimal conditions, which subsequently transform into chains of coccocid cells.

Mycoplasmas are the smallest and simplest free-living organisms known. Mycoplasmas are not obligatory intracellular microorganisms and are usually found extracellularly, but are often found intracellularly in the infected tissues (Mycoplasma, Eds. Wolfgang, J. J., Willette, H. P., Amos, D. B., Wilfert, C. M., Zinsser Microbiology 19th Ed. 1988, Appleton and Lange, 617-623). The term mycoplasma apparently was first used by B. Frank in 1889 (Frank B., Dent. Bot. Ges., 7, 359 (1889) and Krass, C. J. et al., J. Infect. Dis. 23, 62 (1973)). Frank, after careful microscopic observation, began writing about invasion of plants (legume) by these microorganisms and stated: "the characteristic character of the protoplasm in the cortical cells arising from infection, I will designate as mycoplasma". Later, he had more explicitly defined mycoplasma as a mixture of small fungus-like microorganisms and cell protoplasm (Frank, B., Landwirt. Jähr. 19, 523 (1890)). The description reflected the difficulty of differentiating this unique microorganism from the infected host cells morphologically.

Even today with electron microscopy, it is still often difficult to differentiate the mycoplasmas from the cellular protoplasmic processes or the subcellular organelles of the infected host, because ultrastructurally, these microorganisms have protoplasm-like internal structures and are bounded by only an outer limited membrane (unit membrane) without a cell wall. Thus, there have been few electron microscopic studies of mycoplasmas identified directly in the infected tissues of animals or humans.

It has been reported that ultrastructural examination of infected tissues has failed to localize the microbe, even in tissues where very high titers (>10⁷/gm) of microorganisms were recovered in culture (Elizan, T.
S. et al., Pro. Soc. Exp. Biol Med. 139, 52 (1972) and Schwartz, J. et al., Pro. Soc. Exp. Biol Med. 139, 56 (1972)). Therefore, morphologically, the microbe might be mimicking certain normal cellular or subcellular structures in the infected host tissues and preventing direct identification.

In addition to the natural difficulty of morphological differentiation between the microorganisms and the proplasm of infected cells, the often poorly preserved formalin-fixed clinical materials present further limitations to any attempt to directly visualize mycoplasma organisms in the tissues.

DESCRIPTION OF THE BACKGROUND ART

Acquired Immune Deficiency Syndrome (AIDS) is a devastating disease that has afflicted over 70,000 people worldwide (AIDS Weekly Surveillance Report—United States, Centers for Disease Control, Aug. 29, 1988). The disease is clinically characterized by a set of typical syndromes which manifests itself by the development of opportunistic infections such as pneumocystic carinii pneumonia (PCP), toxoplasmosis, atypical mycobacteriosis and cytomegalovirus (CMV). Further characteristics of the AIDS associated syndromes are the clinical manifestation of neuropsychiatric abnormalities, of AIDS encephalopathy (Naura, B. A., et al., Ann. Neurol 19, 517 (1986)), kidney failure of AIDS nephropathy, heart failure of AIDS cardiomyopathy infections and certain uncommon malignancies such as Kaposi's sarcoma or B-cell lymphoma (Durack, D. T., N. Eng. J. Med. 305, 1465 (1981); Reichert, C. M., et al., Am. J. Path. 112, 357 (1983); Ziegler, J. L., et al., N. Eng. J. Med. 311, 565 (1984)).


However, the establishment of an animal model of AIDS by HTLV-III-LAV injection has not been successful. Gajdusek, D.C., et al., Lancet I, 1415 (1984). The chimpanzee is the only primate other than man found to be susceptible to infection by HTLV-III-LAV. However, overt AIDS manifested by the development of opportunistic infections and/or unusual malignancies has not yet been seen, despite evidence for persistent infection and/or viremia in experiments on this species. Gajdusek, D.C., et al., Lancet I, 55 (1985). Thus, the human retroviruses have not fulfilled Koch's postulates, i.e., producing transmissible AIDS-like diseases in experimental animals. HTLV-III/LAV is not associated with the usual malignancies such as B-cell lymphoma and Kaposi's sarcoma, commonly found in patients with AIDS. Shaw, G. M., et al., Science 226: 1165–1171, 1984; Delli Bovi, P. et al., Cancer Research, 46: 6333–6338, 1986; Groopman, J. E., et al., Blood 67: 612–615, 1986. Furthermore, HIV infected patients often show a wide variation in times of disease incubation and speed of disease progression. It is not known whether any specific infectious agent other than HIV can be responsible for the complex pathogenesis often seen in this disease. One such candidate, initially identified as a virus or virus-like infectious agent in parent application Ser. No. 265,920 has now been discovered to be the mycoplasma M. fermentans (incognitus strain).

Although a viral etiology of developing these malignancies has long been suggested, conventional approaches for isolating infectious viral agents have not been fruitful. The presence of a transforming gene or transforming genes (oncogenes) has been associated with Kaposi's sarcoma (Lo. S., et al., Am. J. Path., 118, 7 (1985)). A transformant carrying the transforming gene can cause tumors in mice. However, there is no further characterization of this transforming gene except for the presence of human repetitive DNA sequences. The transforming gene has not been shown to be associated with any viral or virus-like agent. An onconege of AIDs Kaposi Sarcoma was similarly identified following DNA transfection into NIH/3T3 cells and was later characterized in detail (Delli Bovi O. et al., Proc Natl Acad Sci 84, 5660 (1987) and Delli Bovi P. et al., 50, 729 (1987). The oncogene was found to be a rearranged human protooncogene of the fibroblast growth factor (FGF) family.

SUMMARY OF INVENTION

The present invention relates to a novel strain of the mycoplasma M. fermentans which has been isolated from Kaposi's sarcoma of a patient with AIDS. This novel strain of mycoplasma has been designated the incognitus strain of M. fermentans or M. fermentans incognitus. The invention further relates to the use of this incognitus strain of M. fermentans as well as other strains of M. fermentans with homologous antigenic determinants for the detection of specific antibodies in sera of human patients and animals, and for vaccines against mycoplasmas. The invention also relates to antibodies, including monoclonal antibodies, to M. fermentans incognitus and to homologous antigenic determinants of M. fermentans and their use in detecting M. fermentans incognitus antigens in the infected tissue of human patients and animals. The invention further relates to sequencing the DNA of the M. fermentans incognitus and the manufacture of DNA probes based on such sequencing and homologous sequences of M. fermentans for use in the direct detection of the unique DNA sequences in the tissues of human patients and animals.

The present invention further relates to the detection of the presence of M. fermentans incognitus in patients which are HIV-positive or have other acute fulminating systemic disease as an indication of the prognosis of the disease, which can be used to determine the appropriate therapy regimen. The presence of M. fermentans incognitus is determined as described above.

The M. fermentans incognitus DNA is detected in the spleen, liver, brain, lymph nodes, kidney, placenta, lungs, adrenal glands, heart and peripheral blood mononuclear cells of patients with AIDS, or from Kaposi's sarcoma tissue from patients with AIDS. The M. fermentans incognitus DNA is capable of transfecting and transforming NIH/3T3 cells. M. fermentans incognitus is a transmissible virus-like infectious agent in cell cultures, experimental animals and humans. The DNA of the transformants does not contain human repetitive
DNA sequences. Two transformants are identified as Sb51 and Kb43. These transformants are persistently infected by the *M. fermentans* incognitus. *M. fermentans* incognitus is then isolated from the transformants.

The majority of *M. fermentans* incognitus cells have a size of about 140 nm to about 280 nm, with an overall range of 100–900 nm. Introduction of *M. fermentans* incognitus into nude mice and immunocompetent mice (Balb/c) results in a significant morbidity and mortality of the infected animals and the manifestation of many symptoms such as B-cell tumor, spindle cell tumor or immunodeficiency. Similar diseases are transmitted from animal to animal by introduction of infected tissues.

*M. fermentans* incognitus was also found to infect non-human primates (monkeys). *M. fermentans* incognitus antigens were identified in the infected monkey’s sera, and *M. fermentans* incognitus DNA was found in DNA isolated from tissues of the infected monkeys.

*M. fermentans* incognitus and other strains of *M. fermentans* having homologous antigens are capable of detecting antibodies in sera of patients with AIDS, ARC, or non-AIDS patients with this mycoplasmal infection. Any method for detecting an antigen-antibody reaction may be utilized, including enzyme-linked immunosorbent assay (ELISA), immunoradiometric assay, direct and indirect immunofluorescent assay, Western blot technique, and the like. In addition, *M. fermentans* incognitus-specific antibodies (as well as antibodies to homologous antigens of other *M. fermentans* strains) are raised in experimental animals or developed in monoclonal antibodies which are capable of detecting *M. fermentans* incognitus-related antigens in infected tissues. Furthermore, the probes having *M. fermentans* incognitus-specific or homologous *M. fermentans* DNA sequences can be used in the direct detection of *M. fermentans* incognitus DNA in infected tissues, or specific *M. fermentans* incognitus or homologous *M. fermentans* DNA sequences can be used in the polymerase chain reaction (“PCR”) to identify *M. fermentans* incognitus DNA in infected tissues. Since antibodies or antisera are successfully raised against *M. fermentans* incognitus, the *M. fermentans* incognitus or homologous antigens of *M. fermentans* antigens can be utilized to prepare vaccines which may be used to protect animals, including humans, against infection by *M. fermentans* incognitus or other mycoplasmas.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1A shows an electron photomicrograph of *M. fermentans* incognitus.

FIG. 1B shows an electron photomicrograph of *M. fermentans* prototype strain (PG18).

FIG. 1C shows the colony morphology of *M. fermentans* incognitus.

FIG. 1D shows the colony morphology of the prototype strain (PG18) of *M. fermentans*.

FIG. 2A shows antigenic comparison of *M. fermentans* incognitus, *M. fermentans* and other human mycoplasmas in immunoblots immunostained with rabbit antiserum raised specifically against *M. fermentans* incognitus.

FIG. 2B shows mycoplasmas in immunoblots immunostained with mule antiserum raised specifically against *M. fermentans*.

FIG. 3 shows a comparison of DNA homology and restriction patterns between *M. fermentans* incognitus and other human mycoplasmas. The samples were probed with A) pst-8.6, B) psb-2.2, C) RS48, D) MI-H 3.3, E) cDNA clone of *E. coli* rRNA.

FIG. 4A shows direct immunofluorescence staining of *M. fermentans* incognitus using FITC conjugated monoclonal antibody D81E7 (X900).

FIG. 4B shows direct immunofluorescence staining of *M. fermentans* using FITC conjugated monoclonal antibody D81E7 (X900).

FIG. 5A shows the genetic map of a repetitive segment of a 2.2 Kb Eco RI fragment of *M. fermentans* incognitus.

FIG. 5B shows the nucleotide sequence of a repetitive segment of a 2.2 Kb Eco RI fragment of *M. fermentans* incognitus.

FIG. 5C shows the genetic map of a repetitive segment of a 2.2 Kb Eco RI fragment of *M. fermentans* incognitus.

FIG. 6 shows the analysis of repetitive elements following probing with A) psb-2.2 and B-K of FIG. 5A.

FIG. 7A shows detection of *M. fermentans* from urine specimens following PCR stained with ethidium bromide.

FIG. 7B shows detection of *M. fermentans* from urine specimens following PCR stained with Probe RU006.

FIG. 8A shows detection of *M. fermentans* incognitus from urine specimens following PCR stained with ethidium bromide.

FIG. 8B shows detection of *M. fermentans* incognitus from urine specimens following PCR stained with Probe RU006.

FIG. 9 shows analysis of genomic DNA from various strains or isolates of *M. fermentans*.

FIG. 10A shows an electron micrograph of thin sections of *M. fermentans* incognitus cells in the cytoplasm of degenerating Sb51 cells.

FIG. 10B shows an electron micrograph of membrane bound *M. fermentans* incognitus.

FIG. 10C shows an electron micrograph of a partially disrupted *M. fermentans* incognitus at high magnification.

FIG. 11 shows a graph of body weight of monkeys over time, after inoculation with *M. fermentans* incognitus.

FIG. 12A shows immunocytochemical staining of Sb51 cells with non-AIDS serum.

FIG. 12B shows immunocytochemical staining of NIH/3T3 cells with AIDS serum.

FIG. 12C shows immunocytochemical staining of Sb51 cells with AIDS serum.

FIG. 13 shows the immunocytochemical staining of the subcapsular cortical sinus of a lymph node from a patient with AIDS.

FIG. 14 shows the immunohistochemistry of the midbrain of the brain stem of a patient with AIDS.

FIG. 15A shows blotted filters of DNA from Sb51 cells and control NIH/3T3 cells probed with psb-8.6.

FIG. 15B shows blotted filters of DNA from Sb51 cells and control NIH/3T3 cells probed with psb-2.2.

FIG. 16 shows blotted filters of digested DNA from Sb51 cells, control NIH/3T3, cells, cell-free *M. fermentans* incognitus transmission in NIH/3T3 cells and DNA of partially purified *M. fermentans* incognitus probed with psb-8.6.

FIG. 17A shows a sucrose gradient banding of *M. fermentans* incognitus.

FIG. 17B shows DNA and antigen dot blots analysis of sucrose gradient-banded *M. fermentans* incognitus in
which the blot was probed with $^{32}$P in a labeled insert fragment of psb-8.6.

FIG. 18A shows DNA and antigen dot blot analysis of sucrose gradient-banded *M. fermentans* incognitus in which immunochemical staining using pre-immunized rabbit serum was performed.

FIG. 18B shows DNA and antigen dot blot analysis of sucrose gradient-banded *M. fermentans* incognitus in which immunochemical staining using post-*M. fermentans* incognitus immunization rabbit antisera was performed.

FIG. 19A shows Southern blot hybridizations to compare *M. fermentans* incognitus DNA to DNA from known human herpes viruses, vaccinia virus, MCMV and HVS. The samples were probed with A) HSV-1 pHSV-106.

FIG. 19B shows the Southern blot of FIG. 19A using B) VZV pEco A.

FIG. 19C shows the Southern blot of FIG. 19A using C) EBV pBam W.

FIG. 19D shows the Southern blot of FIG. 19A using D) CMV pCMH-35.

FIG. 19E shows the Southern blot of FIG. 19A using E) HBLV pZVH-70.

FIG. 19F shows the Southern blot of FIG. 19A using F) Vaccinia pEHe-1.

FIG. 19G shows the Southern blot of FIG. 19A using G) MCMV pAMB-25.

FIG. 19H shows the Southern blot of FIG. 19A using H) HVS p t 7.4.

FIGS. 20A and 20B shows DNA amplification analysis of various tissue DNA isolated from patients with AIDS and control subjects without AIDS.

FIG. 21A shows *M. fermentans* incognitus-induced histopathological changes of fulminant necrosis in the spleen of a patient without AIDS dying of an acute systemic disease.

FIG. 21B shows the advancing margin of FIG. 21A.

FIG. 21C shows *M. fermentans* incognitus-induced histopathological changes of fulminant necrosis in the lymph node of a patient without AIDS dying of an acute systemic disease.

FIG. 21D shows *M. fermentans* incognitus-induced histopathological changes of fulminant necrosis in the adrenal gland of a patient without AIDS dying of an acute systemic disease.

FIG. 22A shows the immunohistochemistry of *M. fermentans* incognitus-induced necrotizing lesions in the spleen using *M. fermentans* incognitus-specific rabbit antisemum.

FIG. 22B shows the margin of microsis of FIG. 22A.

FIG. 22C shows the immunohistochemistry of *M. fermentans* incognitus-induced necrotizing lesions in the lymph node using *M. fermentans* incognitus-specific rabbit antisemum.

FIG. 22D shows the peripheral zone of necrosis of FIG. 22C.

FIG. 22E shows the immunohistochemistry of *M. fermentans* incognitus-induced necrotizing lesions in the adrenal gland using *M. fermentans* incognitus-specific rabbit antisemum.

FIG. 23A shows in situ hybridization for *M. fermentans* incognitus nucleic acids in the necrotizing lesions of splenic tissue in the peripheral zone around necrosis.

FIG. 23B shows a higher magnification of FIG. 23A.

FIG. 23C shows an area of differing necrosis in splenic tissue.

FIG. 23D shows an area of differing necrosis in splenic tissue.

FIG. 24A shows an electron micrograph of the margin of necrosis of an adrenal gland highly positive for *M. fermentans* incognitus-specific antigens.

FIG. 24A shows a higher magnification of FIG. 24A.

FIG. 24B shows an electron photomicrograph of the peripheral zone of necrosis in lymph node highly positive for *M. fermentans* incognitus-specific antigens.

FIG. 24B shows an electron photomicrograph of the peripheral zone of necrosis in lymph node highly positive for *M. fermentans* incognitus-specific antigens.

FIG. 24B shows a higher magnification of FIG. 24A.

FIG. 25A shows analysis and comparison of DNA restriction patterns of VIa and *M. fermentans* incognitus probed with psb-8.6.

FIG. 25B shows analysis and comparison of DNA restriction patterns of VIa and *M. fermentans* incognitus probed with psb-2.2.

FIG. 26A shows the immunohistochemistry of thymic tissues derived from patients with AIDS.

FIG. 26A shows a higher magnification of FIG. 26A.

FIG. 26B shows the immunohistochemistry of thymic tissues derived from patients with AIDS.

FIG. 26B shows a higher magnification of FIG. 26A.

FIG. 26A shows an electron micrograph of an AIDS thymus immunostained positively for *M. fermentans* incognitus-specific antigens showing mononuclear lym phohistiocytes.

FIG. 27B shows an electron micrograph of an AIDS thymus immunostained positively for *M. fermentans* incognitus-specific antigens showing mononuclear lymphohistiocytes.

FIG. 27B shows an electron micrograph of an AIDS thymus immunostained positively for *M. fermentans* incognitus-specific antigens showing mycoplasma-like particles.

FIG. 27B shows an electron micrograph of an AIDS thymus immunostained positively for *M. fermentans* incognitus-specific antigens showing mycoplasma-like particles.

FIG. 28 shows immunospecific reactive cells from patients with AIDS using monoclonal antibody C42H10.

FIG. 28B shows the immunohistochemistry of livers from patients with AIDS using monoclonal antibody C42H10.

FIG. 28B shows the immunohistochemistry of livers from patients with AIDS using a non-specific monoclonal antibody.

FIG. 28B shows the immunohistochemistry of livers from patients with AIDS using monoclonal antibody C42H10.

FIG. 29A shows an electron micrograph of AIDS liver immunostained positively for *M. fermentans* incognitus-specific antigens at low magnification.

FIG. 29B shows an electron micrograph of AIDS liver immunostained positively for *M. fermentans* incognitus-specific antigens at low magnification.
FIG. 30B shows the immunohistochemistry of a brain derived from a patient with AIDS using monoclonal antibody C42H10.

FIG. 30C shows the immunohistochemistry of a brain derived from a patient with AIDS using a non-specific monoclonal antibody.

FIG. 30D shows the immunohistochemistry of a brain derived from a patient with AIDS using monoclonal antibody C42H10.

FIG. 31A shows electron microscopy of CNS encephalopathy AIDS brains which were histologically unremarkable but immunostained positively for *M. fermentans* incognitos-specific antigens.

FIG. 31B is a higher magnification of FIG. 31A.

FIG. 31C is a higher magnification of FIG. 31B.

FIG. 31D is a higher magnification of FIG. 31C.

FIG. 32A shows the immunohistochemistry of a placenta delivered by a patient with AIDS using monoclonal antibody C42H10.

FIG. 32B is a higher magnification of FIG. 32A.

FIG. 33A shows electron microscopy of an AIDS patient's placenta immunostained positively for *M. fermentans* incognitos specific antigens showing Hofbauer cell.

FIG. 33B shows electron microscopy of an AIDS patient's placenta immunostained positively for *M. fermentans* incognitos specific antigens showing Hofbauer cell.

FIG. 33C shows electron microscopy of an AIDS patient's placenta immunostained positively for *M. fermentans* incognitos specific antigens showing stronal connective tissue.

FIG. 33D shows electron microscopy of an AIDS patient's placenta immunostained positively for *M. fermentans* incognitos specific antigens showing stronal connective tissue.

FIG. 33E shows electron microscopy of an AIDS patient's placenta immunostained positively for *M. fermentans* incognitos specific antigens showing stronal connective tissue.

FIG. 34A shows in situ hybridization for *M. fermentans* incognitos nucleic acid in liver from patients with AIDS.

FIG. 34B shows in situ hybridization for *M. fermentans* incognitos nucleic acid in liver from patients with AIDS.

FIG. 34C shows in situ hybridization for *M. fermentans* incognitos nucleic acid in spleen from patients with AIDS.

FIG. 34D shows in situ hybridization for *M. fermentans* incognitos nucleic acid in spleen from patients with AIDS.

FIG. 35 shows the inhibition of HIV-1-induced syncytium formation by *M. fermentans* incognitos.

FIG. 36A shows the augmentation of cytotoxic effect and inhibition of RT activity in HIV-1 infected A3.01 cells cultures by *M. fermentans* incognitos.

FIG. 36B shows the inhibition of RT activity in HIV-1 infected A3.01 cell cultures by *M. fermentans* incognitos.

FIG. 37A shows continued viral production of HIV-1 and *M. fermentans* incognitos in culture supernatant by ELISA.

FIG. 37B shows continued viral production of HIV-1 and *M. fermentans* incognitos in culture supernatant by electron micrograph.

**DETAILED DESCRIPTION OF THE INVENTION**

A. Definitions

In order to provide a clear and consistent understanding of the specification and the claims, including the scope given to such terms, the following terms as used herein are defined below.

The term “AIDS-like syndrome” is used to describe a set of physiologic conditions or clinical presentations which are commonly used to identify individuals who are suspected of having the disease AIDS, but who have not had confirmation of the disease by blood test. The physiologic conditions are those that are common to individuals with blood test-confirmed AIDS, and include the development of opportunistic infections such as pneumocystic carinii pneumonia (PCP), atypical mycobacterial infection, toxoplasmosis and cytomegalovirus (CMV), the clinical manifestation of progressive weight loss, persistent diarrhea, neuropsychiatric abnormalities of AIDS encephalopathy, kidney failure of AIDS nephropathy, heart failure of AIDS cardiomyopathy, respiratory distress syndrome and infections and uncommon malignancies such as Kaposi’s sarcoma or B-cell lymphoma.

The term “substantial sequence homology” is used to describe substantial functional and/or structural equivalence between sequences of nucleotides or amino acids. Functional and/or structural differences between sequences having substantial sequence homology will be de minimis.

B. Previous Related Applications

The present invention relates to a novel strain of infectious mycoplasma (*M. fermentans* incognitos) isolated from patients with AIDS. The recognition of this pathogen as a mycoplasma has been a slowly evolving process as evidenced by the history of the present specification.

The predecessor patent applications (Ser. No. 875,535, filed Jun. 18, 1986 and Ser. No. 265,920, filed Nov. 2, 1988) identified the subject pathogen as a virus and a virus-like infectious agent (VILLA), respectively. However, continuing study of the pathogen has resulted in the present identification of the pathogen as an infectious mycoplasma. Ser. Nos. 265,920 and 875,535 are incorporated herein by reference.

The presently identified mycoplasma like many other mycoplasmas has many of the characteristics of a virus, which resulted in its identification as such in the original patent application (Ser. No. 875,535, filed Jun. 18, 1986). Further research then showed characteristics which were not typical of classic viruses, thus the characterization as a VILLA in Ser. No. 265,920, filed Nov. 2, 1988. Additional research has now revealed characteristic traits of a mycoplasma as fully explained below.

C. Deposits

A mycoplasma (*M. fermentans* incognitos) according to the invention, in persistently infected cells, is deposited with the American Type Culture Collection under Deposit No. CRL. 9127, deposited on Jun. 17, 1986. *M. fermentans* incognitos, itself is also deposited with the American Type Culture Collection under Deposit No. 53949, deposited on Sep. 29, 1989.

Deposit is for the purpose of completeness but is not intended to limit the scope of the present invention to
the materials deposited since the description as further illustrated by the Examples fully enables the practice of the instant invention. Access to the cultures will be available during the pendency of the patent application to those determined by the Commissioner of Patents and Trademarks to be entitled thereto. All restrictions on availability of said cultures to the public will be removed irrevocably upon the grant of the instant application and said cultures will remain available permanently during the term of said patent 30 years or five years after last request, whichever is longer. Should any culture become nonviable or be destroyed, it will be replaced.

D. Physical Characteristics of *M. fermentans* incognitus

The *M. fermentans* incognitus cell is roughly spherical and about 140-200 nm in diameter, has an outer limiting membrane (about 8 nm thick), and has a buoyant density of about 1.17 g/ml to about 1.20 g/ml in a sucrose gradient. Although *M. fermentans* incognitus could be identified in the nuclei, mature *M. fermentans* incognitus cells are usually seen in the cytoplasm or associated with the plasma membrane of disrupted cytoplasmic cells.

Using Southern blot hybridization analysis, the *M. fermentans* incognitus was distinct from all known members of human herpes virus. *M. fermentans* incognitus was also distinct from vaccinia virus, monkey herpesvirus saimiri (HVS) and mouse cytomegalovirus (MCMV). *M. fermentans* incognitus can be transmitted from culture to culture by cell-free filtrate, after 0.22 micron filtration.

*M. fermentans* incognitus was also found to be distinct from any other known strain of *Mycoplasma*. One unique feature of *M. fermentans* incognitus is its ability to catabolize glucose both aerobically and anaerobically and also to hydrolyze arginine. *M. fermentans* incognitus cannot hydrolyze urea in a biochemical say. When grown in culture, *M. fermentans* incognitus produces a prominent alkaline shift in pH after an initial brief acidic shift. The only other human mycoplasma which is known to metabolize both glucose and arginine is the rarely isolated *M. fermentans* incognitus.

However, the incognitus strain differs from *M. fermentans* in that it appears to be more fastidious in its cultivation requirement and has only been grown in a cell-free modified SP-4 medium. *M. fermentans* also grows in modified SP-4 medium, but at a much faster rate than *M. fermentans* incognitus. Furthermore, *M. fermentans* incognitus can be grown in a variety of commonly used mycoplasma media, whereas *M. fermentans* incognitus cannot.

When grown in the modified SP-4 medium, *M. fermentans* incognitus displays smaller spherical particle size than *M. fermentans* incognitus, and occasional filamentous morphology which is not seen with *M. fermentans* incognitus. Furthermore, *M. fermentans* incognitus forms only irregular and very small colonies with diffuse edges when grown on agar plates. The *M. fermentans* incognitus are cell wall-free and bound by a single triple layered membrane. The average size of an *M. fermentans* incognitus cell is about 180 nm, compared to an average size of about 460 nm for an *M. fermentans* cell.

FIG. 1 shows electron photomicrographs and colony morphology of *M. fermentans* incognitus and *M. fermentans*. Thin sections of concentrated *M. fermentans* incognitus (A) and *M. fermentans* incognitus (B) reveal pleomorphic microorganisms with trilaminar outer unit membrane as designated by the arrows. The bar in 1A and 1B represent 100 nm. *M. fermentans* incognitus (C) and *M. fermentans* (D) formed colonies of apparently different size and morphology after 14 days and 10 days of incubation, respectively. In these figures, the bar represents 50 μm and 20 μm, respectively.

E. Antigenic differentiation of *M. fermentans* incognitus and *M. fermentans*

Further differentiation of *M. fermentans* incognitus from prototype strain of *M. fermentans* (PG18) was displayed by antigenic analysis using both polyclonal and monoclonal antibodies, as well as DNA analysis of sequence homology and restriction enzyme mapping. These analyses showed that the incognitus strain is distinct from all other mycoplasmas, but is most closely related to previously isolated *M. fermentans* strains. *M. fermentans* incognitus was distinguished from *M. fermentans* (PG18 strain) by comparing their specific antigenicity. Polyclonal rabbit antiserum (raised originally against VLIA-sb51) was found to react with both *M. fermentans* (PG18 strain) and *M. fermentans* incognitus, but not with any of the other mycoplasmas tested. However, in the same assay a larger amount of *M. fermentans* (PG18 strain) protein (>0.63 μg) was required to elicit a positive immunochemical response, and the positivity of the reaction rapidly disappeared when the *M. fermentans* (PG18 strain) protein was further diluted. In contrast, a 250-fold to 1000 fold lower concentration of *M. fermentans* incognitus protein still carried a sufficient amount of antigenic determinants to elicit positive reactions in the assay.

In a parallel assay, antiserum raised specifically against *M. fermentans* (PG18 strain) also reacted intensely with *M. fermentans* incognitus. The *M. fermentans* incognitus-specific antiserum reacted as effectively with the antigens of *M. fermentans* incognitus as with the antigens of *M. fermentans* (PG18 strain). There was approximately an equal amount of antigens which could be recognized by the *M. fermentans* incognitus antiserum in each unit of *M. fermentans* (PG16 strain) and *M. fermentans* incognitus proteins. Both *M. fermentans* and *M. fermentans* incognitus proteins could be diluted to 40 ng per well and still elicit a positive reaction.

However, when *M. fermentans* incognitus proteins and *M. fermentans* (PG18 strain) proteins were reacted with monoclonal antibodies raised specifically against *M. fermentans* incognitus, only *M. fermentans* incognitus proteins reacted positively. Six *M. fermentans* incognitus monoclonal antibodies (many with different isotypes) reacted with only *M. fermentans* incognitus, but not with *M. fermentans*. Therefore, *M. fermentans* incognitus carries additional specific antigens which can not be identified in the prototype of *M. fermentans* (PG18 strain).

FIG. 2 shows antigenic comparison of *M. fermentans* incognitus, *M. fermentans* and other human mycoplasmas in immunoblots. Upper blot (2A) was immunostained with rabbit antiserum raised specifically against *M. fermentans* incognitus. Lower blot (2B) was immunostained with mule antiserum raised specifically against *M. fermentans* (PG18 strain). The concentration of mycoplasma protein was dot-blotted decrementally (1:4 dilution) from lane 1 (10 μg) to lane 12 (2.5 pg).

Row A (M. arginini), row B (A. lairdlawii), row C (M. fermentans), row D (M. hominis), row E (M. oale), row F (M. hyorhinis), row G (M. pneumonia), row H (M.
fermentans) and F were immunostained with monoclonal antibodies D81E7, C69H, F93H, B109H, F11C6 and C42H10, respectively. The concentration of mycoplasma protein was dot-blotted decrementally (1:10 dilution) from lane 1 (10 µg) to lane 8 (1 pg). Row a (M. fermentans incognitus) and Row b (M. fermentans).

F. DNA Homology

DNA was isolated from M. fermentans incognitus and ten other species of mycoplasmas (M. orale, M. hyorhinis, M. pneumoniae, M. arginini, M. hominis, M. fermentans, M. genitalium, M. salivarium, U. urealyticum and A. laidlawii) and analyzed on Southern blots, being probed with 32P-labeled cloned M. fermentans incognitus DNA (psb-8.6, psb-2.2) or synthetic oligonucleotide RS48 (SEQ ID NO:1) a M. fermentans incognitus-specific sequence. An additional molecular clone, carrying a 3.3 kilobase insert of M. fermentans incognitus DNA (MI-H 3.3) was also used as a probe.

Although some homology with psb-2.2 was observed in the M. orale genome, no homology with RS48 (the specific DNA sequences occurring at one terminal end of psb-2.2) and no homology with psb-8.6 or MI-H 3.3 were identified in the M. orale genome. Although DNA homology with psb-8.6, psb-2.2, RS48 and MI-H 3.3 were all found in the M. fermentans (PG18 strain) genome, the restriction patterns revealed by these probes were different between M. fermentans (PG18 strain) and M. fermentans incognitus.

Fig. 3 shows a comparison of DNA homology and restriction patterns between M. fermentans incognitus and other human mycoplasmas. The blots were probed with 32P nick-translated psb-8.6 (3A) and psb-2.2 (3B), 32P end-labeled RS48 (3C), 32P labeled MI-H 3.3 (3D) and 32P end-labeled cDNA probe of E. coli ribosomal RNA (3E). Each lane contained 0.2 microgram of EcoRI enzyme pre-digested DNA from Acholeplasma laidlawii (lane 1), M. arginini (lane 2), M. hominis (lane 3), M. hyorhinis (lane 4), M. pneumoniae (lane 5), M. orale (lane 6), M. fermentans (PG18 strain) (lane 7) and M. fermentans incognitus (lane 8). Arrows indicate the positions of standard size marker 23, 9.4, 6.7, 4.4, 2.3, and 2.0 kb, respectively.

Furthermore, there is significant homology between the ribosomal RNA (r-RNA) genes of prokaryotic mycoplasmas and those of Escherichia coli bacteria. The same blot which was consecutively probed with RS48 and MI-H 33 was probed with 32P-labeled cDNA of E. coli or r-RNA, after removing the previously incorporated probe by boiling the filter. The analysis of r-RNA genes revealed both a difference in numbers and size of the hybridization bands with each different species of mycoplasma tested. The EcoRI restriction pattern of the r-RNA genes for M. fermentans incognitus and M. fermentans (PG18 strain) appeared to be identical, but were different from any other mycoplasma tested.

G. Immunofluorescence Staining

Further support for the conclusion that M. fermentans incognitus differs from any other mycoplasma came from a study of direct immunofluorescence staining. An FITC probe was conjugated to the purified M. fermentans incognitus monoclonal antibodies, and again revealed positive staining only in M. fermentans incognitus, but not in M. fermentans (PG18 strain) or six other species of human mycoplasmas. Fig. 4 shows direct immunofluorescence staining of M. fermentans incognitus (A) and M. fermentans (PG18 strain) (B) using FITC conjugated monoclonal antibody D81E7 (X900).

H. M. fermentans incognitus Infection

A high prevalence of M. fermentans incognitus infection has been found in patients with AIDS by using the polymerase chain reaction. The genetic material specific for M. fermentans incognitus has been isolated from spleens, Kaposi’s sarcoma, livers, lymph nodes, peripheral blood mononuclear cells and brains of patients with AIDS.

Furthermore, M. fermentans incognitus infection has been found in previously healthy non-AIDS subjects with an acute fatal disease. The M. fermentans incognitus infection in these patients was directly identified in the necrotizing lesions in lymph nodes, spleens, livers, adrenal glands, heart and brain.

The pathogenesis of M. fermentans incognitus infection is unusual in that despite fulminant tissue necrosis, there is lymphocyte depletion and an apparent lack of cellular immune response or inflammatory reaction in the infected tissues. It is believed that infection of M. fermentans incognitus either has concomitantly caused damage to key components of the hosts’ immune system, or this pathogen has special biological properties which enable it to elude immunosurveillance of the infected hosts.

Coinfection with Mycoplasma fermentans (incognitus strain) enhances the ability of human immunodeficiency virus type-1 (HIV-1) to induce cytopathic effects on human T lymphocytes in vitro. Syncytium formation of HIV-infected T cells was essentially eliminated in the presence of M. fermentans (incognitus strain), despite prominent cell death. However, replication and production of HIV-1 particles continued during the coinfection. Furthermore, the supernatant from cultures coinfected with HIV-1 and mycoplasma may be involved in the pathogenesis of acquired immunodeficiency syndrome (AIDS). Abstract from Science 251, 1074 (1991).

Since the presence of M. fermentans incognitus is most often associated with AIDS and other acute fulminant disease states and more profoundly affects the course of its disease, it can be used to determine the prognosis of these diseases, which information can be utilized for designing therapy regimes. Without being bound by any proposed mechanism, it is believed that antibodies against ORF-1 (see below) may react against CD4+ lymphocytes resulting in an auto-antibody response against CD4 on T cells thus enhancing the cytopathic effects of HIV-1 on T cells.

I. DNA Characteristics of M. fermentans incognitus

M. fermentans incognitus was originally isolated from Kaposi sarcoma tissue of an AIDS patient. The DNA genome of the M. fermentans incognitus is greater than 150 kilobase (kb) pairs and carries repetitive sequences. An 8.6 kb pair cloned probe (psb-8.6) and a 2.2 kb pair cloned probe (psb-2.2) of M. fermentans incognitus detected specific sequences of DNA in Sb51 cells and M. fermentans incognitus infected cells, but not in DNA of uninfected NIH/3T3 cells.

The cloned probes (psb-8.6 and psb-2.2) can be obtained from an EcoRI partial digest of M. fermentans incognitus enriched DNA which is cloned into bacteriophage lambda charon 28. The lambda-recombinant clones are screened by differential plaque hybridization with 32P-labeled DNA derived from gradient banded
M. fermentans incognitus. The insert of the phage clone is then cloned into the EcoRI site of Bluescript KS (M 13-) vector (Stratagene) to produce the cloned probes, psb-8.6 and psb-2.2.

By nucleic acid analysis, the M. fermentans incognitus has been compared with large DNA viruses of the herpes group such as herpes simplex virus type I and II (HSV-I and II), human cytomegalovirus (CMV), Epstein-Barr virus (EBV), Varicella-Zoster virus (VZV) and human B-lymphocytic virus (HBLV) or human herpesvirus-6 (HHV-6), vaccinia virus, Herpesvirus saimiri (HVS) and mouse cytomegalovirus (MCMV). Part of the M. fermentans incognitus genomic DNA has been molecularly cloned. The entire sequence of a cloned M. fermentans incognitus psb-2.2 DNA has been obtained and is shown as SEQ ID NO:2.

To obtain the genetic materials of M. fermentans incognitus, the Kaposi’s sarcoma tissue is minced into small pieces and treated with collagenase. The tissue suspension is then treated with a protease, such as proteinase K. Genetic materials are obtained after phenol extraction, phenol/chloroform/isoamylalcohol extraction, and chloroform/isoamylalcohol extraction. High molecular weight DNA is visibly observed after ethanol precipitation of the genetic materials. The genetic materials are dissolved and contain high molecular weight DNA and RNA of various sizes.

The isolated genetic materials from Kaposi’s sarcoma are utilized to transfect NIH/3T3 cells or other proper recipient cells in accordance with the procedure of Graham, F. L., et al., Virology 52, 456 (1973). In this procedure, the nucleic acid is precipitated with calcium phosphate and incubated with NIH/3T3 cells. The precipitated nucleic acid is removed and the cells trypsinized. The trypsinized cells are reseeded and treated with glycerol before splitting, as described by Copeland, N. G., et al., Cell 16, 347 (1979). The subcultures are fed with Dulbecco’s medium with fetal bovine serum (FBS) and re-fed at three- to four-day intervals.

Foci of morphologically transformed cells become evident in about two weeks. The phenotypical transformation is characterized by rapid overgrowth of the transfected cells which pile up in multilayers and form grossly visible foci. Transformation efficiency is about 0.01-0.02 identifiable foci per microgram of donor nucleic acid. Transformed colonies are harvested after three weeks, and are cultured in monolayers. The DNA of transformants contain human repetitive DNA sequences.

Genetic materials are isolated from the primary transfecteds as previously described, and used to transfect fresh NIH/3T3 cells. Transformation is again seen using the genetic materials with a slightly higher transformation efficiency. This demonstrates that the genetic materials isolated from tissues of AIDS patients contain active transforming elements. This is the first description ever of mycoplasmal DNA transfecting cells.

The nucleotide sequence of the M. fermentans incognitus EcoRI 2.2 kb DNA (plasmid psb 2.2) is shown in SEQ ID NO:2. This plasmid has a segment of unique sequences which occurs repeatedly in the M. fermentans incognitus genome.

By sequence analysis, a genetic element of 1405 base pairs (SEQ ID NO:3) with unique structural characteristics was identified. These unique structural characteristics strongly resemble bacterial insertion sequence (IS) elements. The IS-like element occurs repeatedly in the M. fermentans incognitus genome.

In analyzing the M. fermentans incognitus EcoRI 2.2 kb DNA, one pair of inverted repeats (IR) consisting of 29 bp with seven mismatches was found. These IR are SEQ ID NO:4 (left IR) and SEQ ID NO:5 (right IR). Immediately outside and flanking these 29-bp IR is a 3-bp direct repeat (DR). TTT. The element framed by these two 29-bp IR contains 1405 bp (SEQ ID NO:3). Many pairs of IR that have eight or more contiguous nucleotides are also found within this 1405-bp element. There are two potential stem-and-loop (s&l) structures, L and R, in the element (see FIG. 5) (SEQ ID NO:3). L(AGG = -16.8 kcal/mol) is located exactly at the left terminus of the element, while R (ΔG = -14.4 kcal/mol) is located very near the right terminus. Both of the potential s&l structures are followed by a stretch of T residues pointing toward the interior of the element. These s&l structures with T residues strongly resemble transcription terminators (Rosenberg and Court, Annu. Rev. Genet., 13 319 (1979), which would prevent transcription from the outside into the element (Sylvanen, Annu. Rev. Genet., 18 271 (1984)). The structures may also be responsible for the strong polarity of this element (Grindley and Reed, Annu. Rev. Biochem., 54 863 (1985)). Similar transcription terminators have been found at the termini of several bacterial IS elements. These unique structures are probably maintained for specific benefit of the IS elements and play an important role in the regulation of transposition.

Mycoplasma DNAs are extremely rich in A and T. It has already been shown in the codon usage of ribosomal protein genes of M. capricolum that synonymous nucleotide substitution and conservative amino acid substitution can occur (Muto et al., Nucleic Acids Res., 12 8209 (1984)). It has also been reported that TGA, instead of being a stop codon, is a Trp codon in many species of mycoplasma (Yamao et al., Proc. Natl. Acad. Sci. USA, 82 2306 (1985); Inamine et al., J. Bacteriol., 172 504 (1990)). According to this unique character of codon usages in mycoplasma, three potential ORFs, ORF-1, ORF-2, and ORF-3 (SEQ ID NO:6, 7 and 8, respectively) have been identified in the 2.2-kb DNA. ORF-1 and ORF-2 are located inside the element and ORF-3 is located on the complementary strand 100-bp away from the element.

ORF-1 (SEQ ID NO:6) begins immediately after the s&l structure L at nucleotide 176 and ends at nucleotide 604, and could encode a protein of 143 amino acids (SEQ ID NO:9). There is a possible Shine Delgarno (SD) sequence, AAGGGG (nucleotides 161-166), which precedes the start codon of ORF-1 by 9-bp, and is located inside the s&l structure L (FIG. 5, SEQ ID NO:2 and 3, respectively). There is no consensus sequence for the −10 and −35 promoter regions, however, the left IR may provide a promoter function which has been previously shown in the E. coli IS1 element (Machida et al., J. Mol. Biol., 177 229 (1984)).

ORF-2 (SEQ ID NO:7) begins at nucleotide 1149 and ends at nucleotide 1457, immediately in front of the s&l structure R, and could encode a protein of 103 amino acids (SEQ ID NO:10). There is a promoter-like region which has a −35 region (TTGATT) at nucleotides 1090-1095 and a −10 region (TAGGTT) at nucleotides 1114-1119 located upstream from ORF-2 (FIG. 5, SEQ ID NO:2 and 3, respectively). ORF-3 (SEQ ID NO:8), between nucleotide 1912 and 1637 (on the complementary strand), could encode a 92-amino acids protein (SEQ ID NO:11) (FIG. 5, SEQ ID NO:2 and 3, respectively).
A computer search of the National Biomedical Research Foundation (NBRF) Protein Data Bank has revealed a 40% homology (49% with conservative replacements) between a region of the deduced amino acid sequence of ORF-1 (SEQ ID NO:9; amino acid 101–140) and *Streptococcus pyogenes* Pep M5 protein (amino acids 23–65). The biological function of anti-phagocytosis in this pathogenic bacteria is known to be associated with Pep M5 protein (Fox, *Bacterial Rev.*, 38 57 (1974)). The search also revealed that 75% of the amino acids are identical between a region of the deduced amino acid sequence of ORF-1 (SEQ ID NO:9; amino acid 117–128) and the sequence in the extracellular V4 domain of human T-cell surface glycoprotein CD4 molecule (amino acid 319–329). Another extracellular domain (V1) of the same CD4 molecule is critical for recognition by HIV envelope glycoprotein (Arthos et al., *Cell*, 57 469 (1989)). The significance of the homologies of ORF-1 with Pep M5 protein and the CD4 molecule on human T cells is not clear at this time, but this 75% homology between the amino acid sequence of ORF-1 and CD4 is enough difference to result in the production of antibody to the ORF-1 antigen. However, this antibody may then attack both the ORF-1 antigen and the CD4 receptors due to their similarity.

In a similar analysis, a 43% homology (55% with conservative replacements) between a region of the deduced amino acid sequence of ORF-2 (SEQ ID NO:10; amino acid 18–74) and the deduced amino acid sequence of the putative transposase of *E. coli* IS3 (SEQ ID NO:12; amino acid 189–245) was found. In addition, the ratio of basic to acidic amino acid in protein predicted by ORF-2 is around 2. Thus, this basic protein encoded by ORF-2 highly resembles the *E. coli* putative transposase which is believed to be essential for transpositional recombination (Grindley and Reed, *Annu. Rev. Biochem.*, 54 863 (1985)). No significant homology was found between ORF-3 and sequences in the NBRF Protein Data Bank. Also there is no significant homology between the nucleotide sequence of 2.2-kb DNA (SEQ ID NO:2) and the nucleotide sequences in the GenBank database.

It has been shown that this cloned DNA (psb-2.2; ID SEQ NO:2) contains a unique sequence which occurs more than ten times in the genome of *M. fermentans* incognitus (Lo et al., *Am. J. Trop. Med. Hyg.*, 40 213 (1989)) (also FIG. 6). To precisely define the boundary of this repetitive element, a series of ten oligos, B through K, were synthesized and used as probes. Each probe contained 20–24 nucleotides of a specific sequence from a selected segment in 2.2-kb DNA (FIG. 5). The nt positions of the synthetic oligo, B through K, used as probes to identify the boundary of the IS-like repetitive element in the *M. fermentans* incognitus genome (see FIG. 4) as follows: B (1659–1678), C (1531–1550), D (1514–1533), E (1454–1477), F (1228–1247), G (681–700), H (328–347), I (129–148), J (115–135), and K (44–65) of SEQ ID NO:2. Among the ten oligos, D to I are a series of representative sequences within the 1405-bp IS-like element, and I and D represent sequences within the left and right terminal IR, respectively. B, C, J, and K represent sequences outside the element. Both J and C represent the sequence of the junction areas of the element and actually carry a part of the sequence of the left and right IR, respectively. Each of these synthetic oligo probes was end-labeled with °P and used individually to probe *M. fermentans* incognitus genomic DNA predigested with either EcoRI or HindIII.

The hybridization patterns of multiple bands produced by probes D to I, which carry representative sequences of the various segments in the IS-like element, were essentially the same. In EcoRI digestion, there are eleven identical bands with sizes ranging from 2.20 to 8.90 kb (FIG. 6, D-I, lanes b). When using HindIII digestion, there are twelve identical bands with sizes ranging from 1.95 to 9.10 kb (FIG. 6, D-I, lanes a, b). This pattern of multiple hybridization bands matches exactly with that produced when psb-2.2 DNA is nick-translated and used as a probe (FIG. 6A).

In contrast, the probes B, C, J and K produced a completely different pattern with only a single hybridization band of 2.2-kb in EcoRI digestion or a 1.95-kb fragment in HindIII digestion (FIG. 6B, C, J and K). Probes I (20-mer) and J (21-mer) overlap 7 nucleotides within the left IR; the former produced the typical pattern of multiple bands (FIG. 6I), however, the latter only produced a single band (FIG. 6J).

It was also noted that probes D(20-mer) and C(20-mer) overlap by 3 nucleotides within the right IR; the former produced the typical pattern of multiple bands (FIG. 6D), however, the latter only produced a single band (FIG. 6C). Thus, the 1405-bp IS-like element (SEQ ID NO:3) which is located between nucleotides 129 and 1533, is the repetitive element which occurs more than ten times in the genome of *M. fermentans* incognitus. This finding suggests that the IS-like element is a mobile element. Such mobility suggests the use of this IS-like element as a means for inserting other sequences into other cells (i.e. the IS-like-element can be used as a cloning vector). The presence of multiple gene copies may result from transposition.

The evidence which supports the conclusion that the 1405-bp element is an IS-like element is: (1) the size of the element (1405-bp) being in the range of previously identified bacterial IS elements (800–2500 bp); (2) the presence of 29-bp IR, with seven mismatches located at both of the termini of the element; (3) the presence of a 3-bp DR immediately flanking outside both of the terminal IR; (4) two ORFs (ORF-1 and ORF-2) which could potentially encode two basic proteins; part of the deduced amino acid sequence of ORF-2 being homologous to part of the putative transposase of IS3, and (5) the presence of multiple copies in the genome of *M. fermentans* incognitus. Several other unique structural features found in the 1405-bp element which are also present in bacterial IS elements are: (i) the s&l structure located close to at least one terminus; (ii) the presence of a large number of sequences with properties of IR, and (iii) part (9 bp) of the sequence in one of the terminal IR found again as a repeat sequence (either direct or indirect) near the other terminal IR (see SEQ ID NO:2 & 3).
specific assay using these primers failed to detect some mycoplasmas of the *M. fermentans* species. Ten fg of DNA consistently yielded a positive 160 bp amplified band in DNA isolated from the inocutus strain of *M. fermentans* from a strain (k7) previously isolated from the bone marrow of a patient with leukemia/lymphoma and from other *M. fermentans* strains (MT-2) isolated from contaminated human lymphocyte cultures. A thousand fold higher amount of DNA (10 pg) isolated from the prototype strain of *M. fermentans* (PG-18, and ATCC #19989) as well as DNA from two recent clinical isolates from patients with AIDS tested negative for the diagnostic DNA fragments. Thus, the specific gene arrangement used in this PCR assay was apparently not universally present in the DNA of all *M. fermentans* species.

A more sensitive PCR assay which is able to detect all the different strains or clinical isolates of *M. fermentans*, yet remains highly selective or specific, was then developed based on the presence of multiple copies of an insertion sequence-like (IS-like) genetic element in *M. fermentans*. The actual copy number of the IS-like element found in the genomes of different strains or isolates of *M. fermentans* may vary and range from 5 to more than 10 copies. A new set of primers (RW004 (SEQ ID NO:15) and RW005 (SEQ ID NO:16)) used to produce an amplified fragment of 206 bp in our new PCR assay.

Using the new set of primers and RW006 (SEQ ID NO:17) as a probe, the reaction consistently detected 1 fg of DNA in all *M. fermentans* species tested (FIG. 7) including the prototype strain PG-18 and new clinical isolates from patients with AIDS, whose DNA (up to 10-pg) tested negative in the PCR reaction using the old set of primers. Sensitivity of this newly developed PCR assay was further verified by successfully detecting 1 fg of the *M. fermentans* DNA in the presence of 1 ug of non-specific human background DNA. Specificity of the reaction has also been examined by attempting to amplify the DNAs isolated from other human or non-human mycoplasmas, common tissue culture contaminating mycoplasmas, Gram-positive or Gram-negative bacteria, mouse, monkey and human cell culture and/or tissue. The reaction does not produce the specific 206 bp DNA fragment.

The present study shows that we have developed a highly selective assay to detect *M. fermentans* by PCR with remarkable sensitivity. The assay detects all the different strains and the new clinical isolates of *M. fermentans* that the previous PCR assay using primers RS47 and RS49 failed to detect and appears to be 10 times more sensitive. The limitation of reaction sensitivity per assay for our current PCR is 0.1 to 1 fg *M. fermentans* DNA within a background of 1 ug of human DNA instead of 1 to 10 fg of microbe DNA in our previous PCR assay. Thus, a molecular technique selectively detecting a single microorganism of *M. fermentans* is available.

**K. Infection and Transfection with *M. fermentans* incognitus**

*M. fermentans* incognitus is isolated from the transformants, such as Sb51. In general, Sb51 cell pellets are lysed by freezing and thawing to release *M. fermentans* incognitus particles. The large *M. fermentans* incognitus particles are pelleted through a sucrose barrier and banded in a sucrose isopycnic gradient. The intact *M. fermentans* incognitus particles have a density of about 1.17 to about 1.20.

*M. fermentans* incognitus can be introduced into mice. In general, the *M. fermentans* incognitus isolated from 5×10⁶ Sb51 cells is injected either intravenously or intraperitoneally into six-week-old mice. Nude mice or immunocompetent mice can be infected. Infection of nude mice with *M. fermentans* incognitus results in significant mortality of the infected animals. Many symptoms similarly seen in patients with AIDS are induced by the infected mice. Thus, at necropsy, the infected mice often showed prominent systemic lymphadenopathy, neuropathy or lymphoid depletion with varying degrees of plasmacytosis. Signs of immune deficiency with profound cutaneous infection in some of the animals were noted. Disseminated pruritic skin rashes were also common. There were proliferative lesions of spindle cells in the cutaneous tissue and deep viscera. The immunocompetent mice (Balb/c) infected by *M. fermentans* incognitus were found to be subsequently infected by *Pneumocystis carinii*, which is evidence of the immunodeficient state of these infected animals.

Similar diseases are transmitted from animal to animal by injecting filtrated lysates of spleen, lymph nodes or whole blood from the diseased animals. *M. fermentans* incognitus is also identified in the cytoplasm of the cytopathic cells. Some of the infected mice were found to produce prominent antibody against *M. fermentans* incognitus.

When silver leaf monkeys are inoculated with *M. fermentans* incognitus, the monkeys show wasting syndromes and die within seven to nine months after inoculation. At necropsy, the monkeys do not show evidence of opportunistic infections, acute inflammatory lesions or malignancy. *M. fermentans* incognitus-specific DNA can be directly detected in necropsy tissues of the monkeys, by use of polymerase chain reaction method. *M. fermentans* incognitus infection can be identified in spleen tissue, liver tissue, kidney tissue and brain tissue of the monkeys. Some of the infected monkeys produced antibody to *M. fermentans* incognitus.

**L. Detection of *M. fermentans* incognitus Antigens**

The *M. fermentans* incognitus pathogen is useful for the detection of antibodies in the sera of patients or animals infected with *M. fermentans* incognitus. Some of these patients who are infected with *M. fermentans* incognitus will be patients who have been diagnosed as having AIDS or ARC, Chronic Fatigue Syndrome, Wegener's Disease, Sarcoidosis, respiratory distress syndrome, Kibuchi's disease, autoimmune diseases such as Collagen Vascular Disease and Lupus and chronic debilitating diseases such as Alzheimer's Disease. In one procedure, presistently *M. fermentans* incognitus infected cells are grown in low cell density on sterile glass slides. Sera from suspected patients, and normal subjects are examined in an immunoperoxidase staining procedure such as that described by Hsu, S-M., et al., *Am. J. Clin. Path.* 80, 20 (1983). Using this assay, 23 of 24 sera from AIDS patients showed strong positivity. Serum from the other AIDS patient showed weak positivity. Twenty-six of 30 sera from non-AIDS normal subjects showed no reactivity. The other four non-AIDS patients showed mild to weak reactivity, but much weaker than that of AIDS patients. In addition, some of the sera from experimentally infected animals, as described above, also contained antibodies which...
reacted with the persistently *M. fermentans* infectinus-infected cells in this assay procedure. Similarly, *M. fermentans* infected cells can also be used in this procedure to detect antibodies in sera of infected patients as a result of homologous antigens.

In addition to this procedure, any other procedure for detecting an antigen-antibody reaction can be utilized to detect antibodies to *M. fermentans* infectinus or *M. fermentans* in the sera of AIDS patients or patients with ARC. Such procedures include, but are not limited to, ELISA, Western-blot, direct or indirect immunofluorescent assay and immunoradiometric assay. Such assay procedures for the detection of antibodies in sera of AIDS patients or patients with ARC have been described in U.S. Pat. No. 4,520,113, incorporated herein by reference, which uses HTLV-III/LAV as the antigen. Similar procedures employing *M. fermentans* infectinus or *M. fermentans* can be used. A diagnostic kit for the detection of *M. fermentans* infectinus-specific or *M. fermentans*-specific antibodies can be prepared in a conventional manner using *M. fermentans* infectinus or *M. fermentans*. It is expected that assays utilizing these techniques, especially Western-blot, will provide better results, particularly fewer false-positives.

A final procedure for detecting the presence of *M. fermentans* infectinus or other *M. fermentans* strains in suspected patients is by testing for DNA in conventional methods, preferably using probes based on the sequence of the IS-like element (SEQ ID NO:3). A preferred method is the PCR assay described above.

M. Production of Antibodies to *M. fermentans* infectinus

Antibodies against *M. fermentans* infectinus (or *M. fermentans*) can be produced in experimental animals such as mice, rabbits and goats, using standard techniques. Alternatively, monoclonal antibodies against *M. fermentans* infectinus (or other strains of *M. fermentans*) antigens can be prepared in a conventional manner. Homologous antibodies are useful for detecting antigens to *M. fermentans* infectinus in infected tissues such as lymph nodes, spleen, Kaposi's sarcoma, lymphoma tissue, brain and peripheral blood cells, as well as sera, of patients with AIDS. Any procedure useful for detecting an antigen-antibody reaction, such as those described above, can be utilized to detect the *M. fermentans* infectinus antigens in tissues of patients infected by the mycoplasma.

Rabbit antiserum has been prepared using *M. fermentans* infectinus. The antiserum positively immune stains brain and lymph node tissue from AIDS patients. To produce the antiserum, sucrose gradient banded *M. fermentans* infectinus or any form of concentrated mycoplasma is used with complete adjuvant and administered to rabbits by intraperitoneal and subcutaneous injections at multiple sites. Serum collected from the rabbits is then preabsorbed with NIH/3T3 cells, mouse liver powder and normal human peripheral mononuclear cells isolated from Ficoll-Hypaque gradients. Monoclonal antibodies may also be prepared by conventional procedures.

The antibodies are useful for detecting cells which have been infected by *M. fermentans* infectinus. This capability is useful for the isolation of *M. fermentans* infectinus from other tissues. For example, additional *M. fermentans* infectinus can be isolated by co-cultivating infected tissue from patients with AIDS and a suitable recipient cell line or cells, such as lymphocytes.

The infected cells are assayed or recognized by the antibody, and *M. fermentans* infectinus can be obtained from the infected cells as described above. An affinity column can also be prepared using the antibodies and used to purify the *M. fermentans* infectinus from the infected cell lysate.

N. Vaccines

The *M. fermentans* infectinus pathogen, antigens of *M. fermentans* infectinus or homologous antigens of other *M. fermentans* strains can be utilized as a vaccine in a conventional manner to induce the formation of protective antibodies or cell-mediated immunity. The antigens can be isolated from *M. fermentans* infectinus (or other strains) or can be produced by conventional recombinant DNA techniques. The vaccines are prepared by usual procedures, such as in vitro cell cultures, by recombinant DNA techniques, and by application of the usual and prescribed controls to eliminate bacterial and/or viral contaminations, according to well-known principles and international standard requirements.

Preferably an inactivated, i.e., attenuated or killed, vaccine is utilized. The *M. fermentans* infectinus pathogen is isolated from the infected cells grown in monolayers. *M. fermentans* infectinus is killed by known procedures or modifications thereof, e.g., by the addition of betapropiolactone, Formalin or acetylthelylamine, by ultraviolet radiation, or by treatment with psoralen or psoralen derivatives and long-wavelength ultraviolet light. Alternatively, *M. fermentans* infectinus is attenuated by conventional techniques and isolated.

The vaccine of the invention may contain one or more suitable stabilizers, preservatives, buffering salts and/or adjuvants. The vaccine may be formulated for oral or parenteral administration. Compositions in the form of an injectable solution contain a proper titer of *M. fermentans* infectinus as the active ingredient, and may also contain one or more of a pH adjuster, buffer, stabilizer, excipient and/or an additive for rendering the solutions isotonic. The injectable solutions may be prepared to be adapted for subcutaneous, intramuscular or intravenous injection by conventional techniques. If desired, the solutions may be lyophilized in a usual manner to prepare lyophilized injections.

The dosage of *M. fermentans* infectinus administered will, of course, depend on the mode of administration and the interval of administration. An optimal dose of the active ingredient and an optimal interval of administration can easily be determined by routine preliminary tests known in the art.

The antigens of mycoplasmas such as other strains of *M. fermentans* which share antigenic determinants with *M. fermentans* infectinus can also be used as vaccines to induce the formation of protective antibodies or cell-mediated immunity to *M. fermentans* infectinus. It has been found that antigens of other mycoplasmas share many antigenic determinants with *M. fermentans* infectinus, but lack the pathogenicity of *M. fermentans* infectinus. One such mycoplasma which can then be used in a vaccine against *M. fermentans* infectinus is *M. fermentans*. Other mycoplasmas useful in vaccines against *M. fermentans* infectinus can be determined using conventional techniques for comparing nucleotide sequences for sequence homology.
O. Other Disease States in Which *M. fermentans* incognitus Has Been Implicated

In addition to AIDS, *M. fermentans* incognitus has been implicated in a number of other Disease states including Chronic Fatigue Syndrome, Wegener’s Disease, Sarcoidosis, respiratory distress syndrome, Kikuchi’s disease, autoimmune diseases such as Collagen Vascular Disease and Lupus, and chronic debilitating diseases such as Alzheimer’s Disease. *M. fermentans* incognitus may be either a causative agent of these diseases or a key co-factor in these diseases.

P. Treatment of *M. fermentans* incognitus Infection

*M. fermentans* incognitus is known to be sensitive to a number of antibiotics, including doxycycline, quinolones such as ciprofloxacin, chloramphenicol and tetracycline. Therefore, effective treatment of any of the above implicated diseases should include administration of antibiotics to which *M. fermentans* incognitus is sensitive.

When using the effective antibiotics as the active ingredients of pharmaceutical compositions, the pharmaceutical compositions may be administered by a variety of routes including oral, intravenous, aerosol and parenteral. The amount of active ingredient (antibiotic) necessary to treat an *M. fermentans* incognitus infection will depend on the body weight of the patient, but will usually be from about 0.001 to about 100 mg/kg of body weight, two to four times daily.

Q. Enhancement of HIV-1 Cytotoxic Effects in CD4+ Lymphocytes by *M. fermentans* incognitus

Coinfection with Mycoplasma fermentans (incognitus strain) enhances the ability of human immunodeficiency virus type-1 (HIV-1) to induce cytotoxic effects on human T lymphocytes in vitro. Syncytium formation of HIV-infected T cells was essentially eliminated in the presence of *M. fermentans* (incognitus strain), despite prominent cell death. However, replication and production of HIV-1 particles continued during the coinfection. Furthermore, the supernatant from cultures coinfected with HIV-1 and mycoplasma may be involved in the pathogenesis of acquired immunodeficiency syndrome (AIDS). Abstract from *Science* 251, 1074 (1991). Since the presence of *M. fermentans* incognitus is most often associated with AIDS and other acute fulminant disease states and more profoundly affects the course of its disease, it can be used to determine the prognosis of these diseases, which information can be utilized for designing therapy regimes.

The presence of *M. fermentans* incognitus in patient tissue or cell sample is determined by conventional techniques such as immunoassays, PCR analysis and DNA hybridizations as more fully described herein.

The present invention is further illustrated by reference to the following examples. These examples are provided for illustrative purposes, and are in no way intended to limit the scope of the invention.

**EXAMPLE 1**

Isolation of Genetic Materials from AIDS Patients and Cell Culture

Kaposi’s sarcoma tissue was obtained from a patient with AIDS who died of fulminant *Pneumocystis carinii* pneumonitis. At autopsy, extensive Kaposi’s sarcoma involving skin, both lungs, parietal pleura, gastrointestinal tract, pancreas, liver, kidney and lymph nodes was found. The tissue used to extract genetic material was derived from Kaposi’s sarcoma in the patient’s retroperitoneal lymph nodes, five to six hours after death. Permanent paraffin sections confirmed near-total effacement of lymph node architecture by Kaposi’s sarcoma.

Splenic tissue was obtained from a second patient with AIDS who also died of *P. carinii* pneumonitis. No tumor (i.e., Kaposi’s sarcoma or lymphoma) was identified at autopsy. Paraffin sections of the splenic tissue used to extract genetic material showed congestion and lymphocyte depletion.

The splenic or Kaposi’s sarcoma tissue (1-2 g) was minced into small pieces and treated with collagenase (5 mg/ml) in 1 ml phosphate-buffered saline (PBS) at 37º C. for 15 minutes. The tissue suspension was then treated with proteinase K (250 g/ml) in 10X volume of 150 mM NaCl, 10 mM Tris (pH 7.5), 0.4% SDS, at 65º C. for 30 minutes and at 37º C. for ten hours. Phenol extraction (twice) followed by phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1) extraction were used to purify genetic material. Grossly visible high molecular weight DNA was easily observed after ethanol precipitation. The genetic materials were redissolved in aqueous phase (1 mM Tris, 1 mM EDTA) after overnight air-drying. The recovered genetic materials contained high molecular weight DNA and 30–40% RNA of various size. The procedures of isolating genetic materials from the cultures of the primary transformants and normal human fibroblasts (ATCC, CRL-1521) were similar. The pellets of 10–20 x 10⁶ cells were mixed directly with 10X volume of proteinase K (250 g/ml) in the same buffer without collagenase treatment.

**EXAMPLE 2**

Transfection of NIH/3T3 Cells

The transfection procedures were slightly modified from that of Graham et al., supra. Approximately 30 micrograms of nucleic acid isolated from Kaposi’s sarcoma tissue, splenic tissue, normal human fibroblasts, or salmon sperm were precipitated with calcium phosphate in each 60 mm Petri dish culture (containing about 5 x 10⁵ NIH/3T3 cells). The DNA precipitate was removed after cells were incubated at 37º C. for 12 hours. After an additional 24 hours, each plate of cells was trypsinized and reseeded into four to five 60 mm Petri dishes. The cells received five minutes of 15% glycerol treatment in 10% fetal bovine serum (FBS, Gibco) Dulbecco’s modified Eagle’s medium (DMEM) before the splitting as described by Copeland et al., supra. The subcultures were fed with Dulbecco’s medium with 5% FBS and re-fed with this medium at intervals of three to four days. Foci of morphologically transformed cells became evident in two weeks. Colonies were harvested after three weeks.

NIH/3T3 cells transfected with genetic material derived from both spleen and Kaposi’s sarcoma tissue of AIDS patients produced morphologically transformed colonies which were visible within two weeks. The phenotypical transformation was characterized by the rapid overgrowth of the transfected cells which piled up in multilayers and formed grossly visible foci. Transformation efficiency was approximately 0.01 to 0.02 identifiable foci per microgram of donor nucleic acid. In contrast, no transformed foci were identified in parallel cultures using DNA from salmon sperm or nucleic acid from human fibroblasts. The transformants were recov-
ered from these phenotypically malignant foci after two weeks and cultured in monolayers. Transformants retained their tendency of piling up in multilayers and reached more than three-fold higher cellular density than normal NIH/3T3 fibroblasts.

EXAMPLE 3

Confirmation of NIH/3T3 Cell Transformation

To confirm that transformation of the NIH/3T3 cells was mediated by active transforming genetic elements, the primary transformants' capacity to transmit their malignant phenotypes of rapid cell growth and pile-up (lack of cell-cell contact inhibition) in high cellular density in subsequent cycles of transfection was examined. Thus, a second cycle of transfection, as described above, was performed using genetic material which was isolated as previously described from some of the primary transfectants. A higher efficiency of transformation was observed in the second cycle of the transfection assay (up to 0.05 foci per microgram of donor nucleic acid). These results indicate that genetic materials isolated from spleen and Kaposi's sarcoma tissues of AIDS patients contained active transforming elements that induce malignant transformation of rapid cell growth upon transfection and retransfection of phenotypically normal cells. DNA from first and second stages of transformation clones selected for further studies were then characterized with respect to the presence of human DNA repetitive sequences by probing with 32P nick-translated Blur 8-plasmid. No human repetitive DNA sequences were detected in these transformants.

EXAMPLE 4

Analysis of Transformants

Normal NIH/3T3 and transformant clones were all routinely maintained in monolayer cultures with 10% FBS-supplemented Dulbecco's media. Autoclavable slides (Cell-line Asso. Inc.) were previously sterilized and overlaid with trypsinized cell suspension (1 x 10^5 cells/ml) in square petri dishes. The cultures were incubated at 37°C in a 5% CO₂ incubator for 48 to 72 hours. The culture slides were washed three times with cold PBS, air-dried and stored at 4°C. Immunocytochemistry was performed within two to three days on these stored slides.

The monolayers were scraped directly from the cultures. The cells were harvested by centrifugation of 1,000 rpm for 10 minutes. The cell pellets were fixed overnight at 4°C in 2.5% glutaraldehyde in phosphate buffer and post-fixed with 1% OsO₄. The fixed tissues were then processed by standard methods and embedded in Maraglass 655. The grids with ultra-thin sections were double-stained with uranylacetate and lead citrate. The specimens were then examined under an electron microscope with 60 kv or 100 kv voltage. Negative staining of the virus-like particles in the culture supernatants was performed. Briefly, the particles in the culture supernatant were pelleted through a 5 ml 20% sucrose barrier in SW41 centrifugation tubes, at 40,000 rpm for one hour. The pellets were then resuspended in 1/50 to 1/100 volume of Tris-normal saline (pH 7.4, 0.05M Tris). The suspensions were directly put on formvar coated grids and negatively stained with 2% phosphotungstic acid (PTA) (pH 7.2).

Two of the transformants (Sb51 and Kb43, from different patients) were studied in detail. These two transformants were obtained from the second cycle transfections with genetic materials from Kaposi's sarcoma spleen and tissues, respectively. Sb51 cells persistently infected with M. fermentans incubitus were deposited with the ATCC under No. CRL 9127 under the Budapest Treaty on Jun. 17, 1986. The cells grew in high cellular density with no significant cytopathic changes. However, occasional lytic plaques, with cells showing cytopathic changes, were noted after the transformants reached saturated density. Many physiologic factors, including incubation temperatures and culture media, were found to affect the degree of lytic plaque formation. For example, a reduction in the temperature to 32°C results in higher lytic plaque formation. Sb51 cells tended to pile-up in a monolayer culture. Foci of rapid cell overgrowth and pile-up into multilayered layers can best be appreciated under low-power light microscopy with a dark background. Cytopathic changes commonly occurred in the centers of the high cell density foci. Detachment of the cytolytic cells in the center of hyperplastic foci was evident. There were prominent cytopathic effects among the densely-packed cells on the peripheral edges of the lytic plaque. These cells rounded up and appeared smaller in size with a shrunken configuration.

The monolayers of Sb51 and Kb43 which showed significant cytopathic changes in at least 30% of cells were examined by electron microscopy.

In those cells undergoing cytopathic changes numerous M. fermentans incubitus cells were seen, mainly in the cytoplasm of disrupted cells. Early cytopathic changes showing nuclear chromatin condensation and margination was seen at 15,000X magnification. Accumulation of M. fermentans incubitus nucleocapsids within the nucleus is prominent. Numerous M. fermentans incubitus particles of different maturation stages were seen in the cytoplasm at 45,800X magnification. Most of the mature M. fermentans incubitus cells in the cytoplasm are lined up along the plasma membrane while others are free. The M. fermentans incubitus cells were roughly spherical enveloped particles of heterogenous sizes. The majority of mature M. fermentans incubitus cells were 140-280 nm, with an overall range of 100-900 nm. The intact M. fermentans incubitus particle had a well-defined outer limited membrane about 8 nm thick and tightly packed internal nucleocapsids. Occasionally, the nucleocapsids were seen to condense into compact cores inside the M. fermentans incubitus cell. Although the M. fermentans incubitus outer envelope was well-defined and thick, it was not rigid. Elongated, ovoid, and pleomorphic forms with protrusions were not uncommonly identified among the M. fermentans incubitus cells (at 45,800X magnification).

To further confirm the ultrastructure and morphology of M. fermentans incubitus, the unsectioned M. fermentans incubitus were examined by pelleting M. fermentans incubitus particles from Sb51 and Kb43 culture supernatants through a 20% sucrose gradient barrier. The particles were resuspended in Tris-normal saline at 1/100 of original volume. The precipitated particles were directly examined under electron microscopy following negative stainings with PTA. Some preparations of the intact M. fermentans incubitus particles were briefly fixed with 0.5% Formalin to preserve the M. fermentans incubitus morphology as well as to avoid possible infectious problems in the laboratory. The negative staining preparations of M. fermentans...
incognitus usually revealed more surface detail together with their internal structure. There was some heterogeneity in both particle size and shape. Some M. fermentans incognitus cells often appeared to be elongated or had irregular bulging protrusions (when viewed at 101,800× magnification). The internal component consisted of strands arranged more or less parallel to each other and to the long axis of the particle. The internal nucleocapsid strands appeared to be better preserved in the particles fixed with low concentrations of Formalin. The well-defined envelope revealed inconspicuous spikes on the external surface. At high magnification (370,000X), M. fermentans incognitus demonstrated complex membranous envelopes. The released nucleocapsids appear to be uncoiled.

**EXAMPLE 5**

PCR Assay for M. fermentans incognitus

An assay of urine sediments prepared in Example 6 is illustrative of a PCR assay. The amplification of selective DNA sequences was performed with thermostable Taq DNA polymerase (Native Taq; Perkin Elmer Cetus, Norwalk, Conn.) (10) in the automated Perkin-Elmer Cetus DNA thermal cycler (Norwalk, Conn.). One ml of urine sediment prepared and filtered as described in Example 6 was first centrifuged at 1,500 x g for 15 min. Nine-hundred ul of the supernatant was removed. Proteinase K was added to the remaining 100 ul sample (final concentration of 200 µg/ml) and the sample was digested at 56° C for 2 hrs. Before PCR analysis the digested samples were heated at 95° C for 10 min. Each 10 ul urine sediment sample to amplified was adjusted to a total volume of 160 ul with PCR buffer containing a final concentration of 50 mM KCl, 20 mM Tris-HCl (pH 8.3), 1 mM MgCl₂, 0.001% gelatin, each primer (RW004 (SEQ ID NO:15) and RW005 (SEQ ID NO:16) (R. Y-H Wang et al., Abstr. Gen. Meet. Am. Soc. Microbio. 1991, G-5, p. 134) at 0.5 µM, each dNTP at 250 µM and 2.5 U of Taq DNA polymerase. It has been found that these primers are preferred over the RS47 and RS49 primers used in PCR assays below (Example 16 and 19). The samples were overlaid with 3 drops of mineral oil (50 ul). Samples were denatured at 94° C for 35 sec, annealing of primers at 56° C for 45 sec and extension at 72° C for 1 min. The annealing time was increased by one sec/cycle during the amplification. After the final cycle, the annealing time was increased to 5 min, followed by extension for 5 min. Twenty ul aliquots from each amplified sample were removed and analyzed on a 6% polyacrylamide gel in 1× Tris-borate-EDTA buffer (Maniatis et al., *Molecular Cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982)); the gels were stained with ethidium bromide and the DNA visualized by UV fluorescence. The fractionated DNA was electroblotted onto a Zeta-Probe membrane (BioRad, Richmond, Calif.) at 100 volts for 2 hrs., in 0.5× Tris-acetate-EDTA buffer (Maniatis et al., supra), followed by denaturation and fixation in 400 mM NaOH, 2 mM EDTA for 10 min. at room temperature. The Zeta-Probe membrane was rinsed 3 times with 2× SSC in 20 mM Tris-HCl (pH 7.5) and air dried for 10 min. Prehybridization was carried out in 30% formamide, 4× SSC, 5× Denhard’ts, 20 mM Tris-HCl, (pH 7.5), 2 mM EDTA, 1% SDS and 350 µg/ml of denatured salmon sperm DNA at 30° C. Hybridization was in the same mixture but containing the oligonucleotide probe RW006 (SEQ ID NO:17) (Wang et al., *Abstr. Gen. Meet. Am.*

**TABLE 1**

<table>
<thead>
<tr>
<th>Sources</th>
<th>Concentration of DNA tested</th>
<th>Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycoplasmas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>M. fermentans</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 19989</td>
<td>1 fg</td>
<td>+</td>
</tr>
<tr>
<td>incognitus strain</td>
<td>1 fg</td>
<td>+</td>
</tr>
<tr>
<td>PG-18</td>
<td>1 fg</td>
<td>+</td>
</tr>
<tr>
<td>K-7</td>
<td>1 fg</td>
<td>+</td>
</tr>
<tr>
<td>MT-2</td>
<td>1 fg</td>
<td>+</td>
</tr>
<tr>
<td>and nine clinical isolates</td>
<td>1 fg</td>
<td>+</td>
</tr>
<tr>
<td><strong>M. hominis</strong> (ATCC 15488)</td>
<td>1 ng</td>
<td>-</td>
</tr>
<tr>
<td><strong>M. orale</strong> (ATCC 23714)</td>
<td>1 ng</td>
<td>-</td>
</tr>
<tr>
<td>and one clinical isolate</td>
<td>1 ng</td>
<td>-</td>
</tr>
<tr>
<td><strong>M. salivarius</strong> (ATCC 23064)</td>
<td>1 ng</td>
<td>-</td>
</tr>
<tr>
<td>and two calinal isolates</td>
<td>1 ng</td>
<td>-</td>
</tr>
<tr>
<td><strong>M. buccale</strong></td>
<td>1 ng</td>
<td>-</td>
</tr>
</tbody>
</table>

*Soc. Microbiol.* 1991, G-5. p. 134) which was 5’end labeled with 32P-ATP, and was conducted overnight at 30° C. After hybridization the membrane was washed at 45° C in 2× SSC, 0.5% SDS four times (30 min. each). Forty-three urine sediments obtained from 40 HIV positive patients and 50 urine sediments obtained from HIV negative healthy control individuals were tested for the presence of M. fermentans specific DNA sequences by using the PCR assay. Primer pairs of synthetic oligonucleotides, designated RW004 (SEQ ID NO:15) and RW005 (SEQ ID NO:16) containing specific sequences within the insertion sequence (IS)-like genetic element found in multiple copies in M. fermentans mycoplasmas were used to amplify a 206 bp segment of the IS-like DNA. Ten of 43 urine sediments obtained from HIV positive patients with varying stages of AIDS disease, tested positive for the presence of M. fermentans DNA. In contrast, none of the 50 urine sediments obtained from HIV negative non-AIDS controls tested positive. FIG. 8 shows the PCR results of representative samples from HIV negative controls (FIG. 8, lanes b and c) and HIV positive patients’ urine sediments (FIG. 8, lanes d-m). Lane n contained one femtogram M. fermentans incognitus DNA diluted into one micromgram of human placental DNA and lane o contained pUC18 DNA digested withMspI, serving as size markers. A distinct band could be observed in the ethidium bromide stained gel at a position corresponding to the 206 bp fragment amplified in M. fermentans control DNA (FIG. 8A, lane n), and in positively amplified AIDS patients' urine sediments (FIG. 8A, lanes d-f, h, k and l). The RW006 (SEQ ID NO:17) probe hybridized strongly to all positively amplified samples (FIG. 8B, lanes d-f, h, k, l, and n).

Using a similar procedure, M. fermentans species including the prototype strain PG-18 and new clinical isolates from patients with AIDS, which had tested negative in previous PCR reactions were analyzed in a PCR reaction using RW004 (SEQ ID NO:15) and RW006 (SEQ ID NO:16) as primers. The assay consistently detected 1 fg of DNA in all species (FIG. 7). Specificity of the reaction has also been examined by attempting to amplify the DNAs isolated from other human or non-human mycoplasmas, common tissue culture contaminating mycoplasmas, Gram-positive or Gram-negative bacteria, mouse, monkey and human cell culture and/or tissue. The reaction does not produce the specific 206 bp DNA fragment (Table 1).
TABLE 1-continued

SPECIFICITY OF PCR FOR *M. FERMENTANS* USING UNIQUE SEQUENCES WITHIN THE IS-LIKE GENETIC ELEMENT

<table>
<thead>
<tr>
<th>Sources</th>
<th>Concentration of DNA tested</th>
<th>Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. pneumoniae</em> (ATCC 15531)</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. genitalium</em> (ATCC 33530)</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. argini</em> (ATCC 23838)</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. prun</em>*</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. alvi</em>*</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. moos</em>*</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. suavi</em>*</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. ioea</em>*</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. arthridis</em>*</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>M. hyorhin</em> (ATCC 17981)</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>Acholeplasma laidi</em> (ATCC 23206)</td>
<td>1 ng</td>
<td>—</td>
</tr>
<tr>
<td><em>Ureaplasma urealyticum</em> (ATCC 27618)</td>
<td>1 ng</td>
<td>—</td>
</tr>
</tbody>
</table>

**Bacteria**

<table>
<thead>
<tr>
<th></th>
<th>Concentration of DNA tested</th>
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</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>1 ug</td>
<td>—</td>
</tr>
</tbody>
</table>

**Mouse**

<table>
<thead>
<tr>
<th></th>
<th>Concentration of DNA tested</th>
<th>Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH/3T3</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Spleen (Balb/c)</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Liver (Balb/c)</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Brain (Balb/c)</td>
<td>1 ug</td>
<td>—</td>
</tr>
</tbody>
</table>

**Monkey**

<table>
<thead>
<tr>
<th></th>
<th>Concentration of DNA tested</th>
<th>Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vero cells (ATCC CCL18)</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Spleen (green monkey)</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Liver (green monkey)</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Brain (green monkey)</td>
<td>1 ug</td>
<td>—</td>
</tr>
</tbody>
</table>

**Human**

<table>
<thead>
<tr>
<th></th>
<th>Concentration of DNA tested</th>
<th>Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCRF-cem (ATCC CCL119)</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>Placenta (nl. delivery) 4X</td>
<td>1 ug</td>
<td>—</td>
</tr>
<tr>
<td>PBMC (nl. donor) 50X</td>
<td>1 ug</td>
<td>—</td>
</tr>
</tbody>
</table>

**EXAMPLE 6**

Direct Isolation of AIDS-associated Mycoplasma From Infected Tissues of AIDS Patients

Urine was collected in sterile containers and concentrated 10-fold by centrifugation (3000 x g for 15 min. at 4°C) and resuspended in 1/10 of the original urine. The resulting urine sediments were diluted 1:10 in modified SP-4 media (Lo et al. 1989a, *Am. J. Trop. Med. Hyg*. 41: 586–600) and then filtered through a 0.22 um filter.

The filtered urine sediments (10 ml), previously diluted in modified SP-4 media, were cultured in 25 cm² tissue culture flasks and also cultured with a further: 1:10 aerobically and in GasPak jars (BBL, Microbiology Systems, Cockeysville, Md.) anaerobically. Flasks showing a color change were subcultured to modified SP-4 agar to confirm the mycoplasma growth. Speciation of various mycoplasma colonies obtained was assayed by immunofluorescence of colonies on agar using species-specific FITC-conjugated antibodies (Del Guidice et al. 1967, *J. Bacteriol*. 93:1205–1209).

Restriction endonuclease cleavage and Southern blot hybridization of genomic DNA from prototype strains and new clinical isolates of *M. fermentans* was carried out basically as previously described (Lo et al. 1989a, supra; Lo et al. 1989b, *Am. J. Trop. Med. Hyg*. 41:213–226). DNA was isolated from cultures of each isolate or strain of *M. fermentans*, purified by standard methods, and digested with either EcoRI or HindIII restriction enzymes (Gibco-BRL, Gaithersburg, Md.). The enzyme digests of NDA, after electrophoresis in 1% agarose, were transferred by electro transfer to Zeta-Probe membrane by the Southern blot method. Each filter was prehybridized in 50% formamide, 4 x SSC, 5 x Denhardt’s, 20 mM Tris-HCl (pH 7.5), 2 mM EDTA, 1% SDS, and 250 ug/ml denatured salmon sperm DNA for at least 4 hrs at 42°C. and hybridized with 32P nick-translated psb-2.2 DNA (Hu et al. 1990). Gene 93:67–72 at 42°C in the prehybridization solution as described, (Lo et al. 1989b, supra). After hybridization the blots were washed at 55°C in 2 x SSC, 0.5% SDS, 10 mM Tris-HCl (pH 7.5) for 120 min. with 4 changes and then washed at 50°C in 0.5 x SSC, 0.1% SDS for 60 min. with 2 changes before autoradiography (Lo et al. 1989b, supra).

*M. fermentans* was isolated and grown in modified SP-4 media from 3 of the AIDS patients’ urine sediments which tested positive in the PCR assay of Example 5. DNA was prepared from cultures of the new clinical isolates and compared with that of representative *M. fermentans* strains in Southern blot analysis. The DNAs were digested with EcoRI (a lanes) and HindIII (b lanes), fractionated in an agarose gel and hybridized with 32P-labeled psb-2.2 (FIG. 9). Lane m is HindIII digested lambda phage DNA used as marker of 23.1, 6.6, 4.4, 2.3 and 2.0 kb, respectively. The new clinical isolates (FIG. 9, D and E) have similar but distinct restriction enzyme patterns from K7 strain (FIG. 9, A) PG18 prototype strain (FIG. 9, B), original *M. fermentans* incognitus (FIG. 9, C) which indicates that they are indeed independent isolates.

*M. fermentans* mycoplasmas were successfully isolated and grown in mycoplasma culture from 3 urine sediments derived from 2 HIV positive individuals (Table 1). Five *Ureaplasma urealyticum* and two *M. hominis* were also isolated from the 43 cultures of AIDS patients’ urine sediments. Fifty urine sediments similarly prepared from age-matched HIV negative healthy controls did not grow *M. fermentans* mycoplasmas. In this study, 23 *Ureaplasma urealyticum* and *M. hominis* were isolated from the 50 control urine sediments (Table 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Species</th>
<th>HIV Positive AIDS Patients</th>
<th>HIV Negative non-AIDS controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. fermentans</em></td>
<td>3/43 (7.0%)†</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td><em>M. fermentans</em></td>
<td>2/43 (4.7%)†</td>
<td>1/50 (2.0%)</td>
</tr>
<tr>
<td><em>U. urealyticum</em></td>
<td>5/43 (11.6%)‡</td>
<td>23/50 (46.0%)</td>
</tr>
</tbody>
</table>

†Number of isolates over number of samples cultured
‡Percentage of isolation

**EXAMPLE 7**

Isolation and Gradient Banding of *M. fermentans* incognitus

Sb51 cells grown as monolayers were briefly trypsinized and pelleted by centrifugation at 1,000 rpm for 10 minutes. The cell pellet was resuspended with an equal volume of Dulbecco’s medium. The cells were lysed by five cycles of freezing and thawing to release the cell-associated *M. fermentans* incognitus particles. The particles were pelleted through a 20% sucrose barrier in a SW41 centrifuge tube by centrifugation at 40,000 rpm for 45 minutes. The particles were resuspended in PBS and banded in a sucrose isopycnic gradient (20% to 60%). Electron micrographs of the *M. fermentans* incognitus cells in the cytoplasm of degenerating Sb51
cells is shown in FIG. 10. The *M. fermentans* incognitus particles were localized at a density of about 1.17 to about 1.20 (FIGS. 10(B) and 10(C)). The *M. fermentans* incognitus particles were directly identified by electron microscopy with PTA negative staining.

**EXAMPLE 8**

Production of Antibodies Against *M. fermentans* incognitus

*M. fermentans* incognitus particles were isolated as described in Example 7 from $5 \times 10^6$ Sb51 cells, and mixed with Freund’s adjuvant. Rabbits were injected with the immunogen twice at a two- to three-month interval. A good antibody response to *M. fermentans* incognitus was obtained after the second immunization.

**EXAMPLE 9**

Infection of Mice by *M. fermentans* incognitus

*M. fermentans* incognitus was isolated as described in Example 7, from $5 \times 10^6$ Sb51 cells, and resuspended in a small amount of PBS. The *M. fermentans* incognitus suspension was injected into either a six-week-old NIH (Nu) male mouse or a six-week-old Balb/c male mouse. The injection was performed either intravenously or intraperitoneally. Sixty percent of the nude mice which received intravenous or intraperitoneal injections of the *M. fermentans* incognitus preparation showed evidence of skin rashes with areas of erythematous changes and conjunctivitis in 10 to 12 days. One animal also showed prominent periorbital edema. These signs disappeared after two to three weeks. All the animals appeared to recover from the acute infection. Two animals then developed pruritic skin rashes after six weeks. These two animals and the other two animals died or became too sick, and had to be sacrificed in three months. Therefore, 40% of the animals did in the first three months following injection. One animal which did not develop recognizable skin lesions showed systemic lymphadenopathy and paralysis. The animal appeared to be wasting and experienced complete paralysis of its hind legs. One animal had several purplish skin lesions which were slightly raised. At necropsy, all lymph nodes in these animals showed lymphocyte depletion. Only very small lymph nodes were identified on gross examination. In contrast, disseminated lymphadenopathy was seen in the inguinal, axillary, cervical, mediastinal and mesentery lymph nodes. The animal also developed hepatosplenomegaly. Histologic sections of the lymph nodes revealed prominent plasmacytosis. Areas of sinus histiocytosis were also noted. The plasma cell effaced normal lymph node architecture and diffusely infiltrated the sinus. Lymph nodes in all the other animals showed lymphocyte depletion. Only small lymph nodes could be identified grossly.

Histologic sections of purplish skin lesions revealed spindle cell proliferation. The spindle cells appeared to infiltrate cutaneous adipose tissue as well as underlying muscles. Extravasation of red blood cells was seen in some areas. Mitotic figures were identified, but not prominent. Histologic examination of the liver of the animal also revealed spindle cell proliferation in the perportal areas. The homogeneous tumor cells exhibited more epithelioid appearance. Numerous red blood cells were trapped in the intercellular slits. Electron microscopic examination of the infiltrating spindle cells in the skin lesions revealed cells with cytopathic changes. An accumulation of *M. fermentans* incognitus nucleocapsids were seen in many of the nuclei, and some in the cytoplasm. The morphology and the characters of these *M. fermentans* incognitus nucleocapsids were similar to those observed in Sb51 cells previously described. Mature *M. fermentans* incognitus cells were also identified in some of the disrupted cells. Both nucleocapsids and *M. fermentans* incognitus cells were often seen in dilated cisternae of smooth endoplasmic reticulum. Electron microscope studies of the perportal spindle cell lesions in the liver similarly revealed prominent infection of *M. fermentans* incognitus.

Balb/c mice infected with the *M. fermentans* incognitus also appeared to be sensitive to the *M. fermentans* incognitus pathogen. Three of seven animals died in the first three months following infection. Two more animals died in the fourth month following infection. None of the control animals showed any disease in four months. Clinical evaluation of skin rashes and lymphadenopathy while these animals were alive was much more difficult. At necropsy, all of the animals were found to be lymphocyte-depleted. The animals had very small lymph nodes and spleens. Lymph nodes were often unrecognizable grossly. The lungs of these animals were found to have severe pneumonia. M-Ag and tolune blue staining revealed *P. carinii*. Therefore, these animals were believed to be severely immunodeficient. Two of the animals who survived for more than four months were found to have antibody in their sera which recognized Sb51 cells but not NIH/3T3 parental cells. Immunoperoxidase reaction of the sera showed positive reactions in both the nuclei and the cytoplasm of Sb51 cells indicating the presence of *M. fermentans* incognitus.

**EXAMPLE 10**

Infection of Non-Human Primates with the *M. fermentans* incognitus

Four silver leaf monkeys (presbytis cristatus) were inoculated (intraperitoneally) with partially purified *M. fermentans* incognitus (see Example 7 above). All four monkeys displayed a wasting syndrome as shown in FIG. 11, and died within seven to nine months. A control monkey which had been inoculated with a preparation derived from normal NIH/3T3 cells did not exhibit the wasting syndrome and did not die during the seven- to nine-month period.

The monkeys were followed daily for signs of illness, and examined once every two weeks for body weight, body temperature and general physical condition. Serial blood samples were also collected every two weeks for blood cell counts and antibody and antigen assays.

Two weeks after *M. fermentans* incognitus inoculation, one monkey showed signs of a flu-like syndrome which persisted for six weeks. This same monkey later developed facial/neck edema (between week 8 and week 12), poor skin tones, and dermatitis associated with alopecia (after week 18). This was the first monkey to succumb, expiring at the 29th week after *M. fermentans* incognitus inoculation. The animal had apparently been afebrile throughout the whole course, except at the time of the 16th week after *M. fermentans* incognitus inoculation.

Body weights of all *M. fermentans* incognitus inoculated monkeys fluctuated. However, a progressive weight loss was noted among these animals in the last 14 weeks of the experiment (FIG. 11). No diarrhea was detected for any of the animals. Two of the monkeys
also had transient lymphadenopathy at 4 to 14 weeks and 4 to 20 weeks after M. fermentans incognitus inoculation, respectively. Three monkeys appeared to have persistent low grade fever in the earlier course of the experiment, but no significant febrile response could be detected in the later stages (the last month). The moribund animals revealed paradoxical pyrexia in the final periods. One monkey revealed signs of tremor, rigidity and imbalance in the terminal stage. The clinical signs strongly suggested a neurological illness. Accurate assessment, however, was hampered by the obvious physical weakness of the animal which may have been due to the prominent weight loss.

At necropsy, no malignant tumor or opportunistic infection could be identified in any M. fermentans incognitus inoculated animal. Histopathology of the lymph nodes obtained from these monkeys revealed features of lymphocyte depletion. There was spindle cell proliferation in the perinodal areas, but typical diagnosis of Kaposis's sarcoma could not be made.

One animal revealed persistent and significant leukocytosis that lasted for about three months (between 16 to 28 weeks after inoculation). In contrast, two other monkeys showed prominent leukopenia in the terminal stage. Differential cell count revealed that their lymphocytes were 448, and 410 per microliter, respectively. Both red blood cell and platelet counts fluctuated. Transient periods of low platelet counts were observed during the course of the study for all animals. However, no animal was thrombocytopenic in the terminal stage.

To study if the M. fermentans incognitus inoculated animals developed an immune response and produced specific antibodies, the serum samples obtained from serial bleedings during the course of the experiment were examined. Sucrose gradient-banded M. fermentans incognitus was used as the antigen in the Western blot antibody analysis. Seroreversions which were defined by definite changes of the immunoreactive patterns and development of new reactive bands on the blot strips after M. fermentans incognitus inoculation, occurred unusually late. Only one monkey had a prominent antibody response, which, however, occurred as late as seven months after M. fermentans incognitus inoculation. Another monkey had a transient antibody response for two months (six months to eight months after M. fermentans incognitus inoculation) which apparently disappeared in the terminal stage, one month before the animal expired. The other two monkeys had a poor and very late immune response which again only occurred in the terminal stage, 4 to 6 weeks before the animals expired. No antibody response could be detected in the control monkey. Estimated molecular weights for the newly developed major protein bands which revealed a positive reaction with the first monkey's sera obtained seven months post M. fermentans incognitus inoculation, were 97, 88, 84, 32.5 and 27.5 kilodaltons, respectively.

M. fermentans incognitus antigens in the animals' sera obtained during the course of the experiment were also measured. By sandwiched radioimmunooassay using rabbit antiserum raised against M. fermentans incognitus antigens, periodic M. fermentans incognitus antigenemia was detected in the three monkeys which failed to produce a prominent antibody response. The first monkey to succumb showed the most prominent, early and persistent M. fermentans incognitus antigenemia.

To further confirm that these animals inoculated with M. fermentans incognitus suffered a fatal systemic infec-

tion by M. fermentans incognitus, DNA obtained from various tissues taken at necropsy was directly examined. In this study, the highly sensitive polymerase chain reaction (PCR) method of selective DNA amplification was used. Primer pairs (RS47 [SEQ ID NO:13]/RS49 [SEQ ID NO: 14]) of synthetic oligonucleotides with M. fermentans incognitus-specific sequences and Taq DNA polymerase were used for 35 reaction cycles of M. fermentans incognitus-specific DNA amplification. The primer pairs RS47/RS49 were previously shown to span the first 160 bp region at one terminal end of M. fermentans incognitus DNA of psb-2.2 (SEQ ID NO:2). The presence of M. fermentans incognitus-specific DNA in the amplified products was confirmed by blot hybridization using synthetic oligonucleotide probe (RS48 [SEQ ID NO:1])' 5' end-labeled with 32P. The typical positive hybridizations for M. fermentans incognitus-specific DNA products revealed diagnostic 160 bp DNA fragments with sequence homology to RS48 (SEQ ID NO:1) representing a central segment of the intervening sequences between RS47 (SEQ ID NO:13) and RS49 (SEQ ID NO:14). In the PCR, M. fermentans incognitus DNA was found in spleen, liver, brain and kidney of the M. fermentans incognitus inoculated animals, but not in the tissues of the control animal.

The necropsy tissues of two monkeys' livers as well as a monkey which appeared to contain the most abundant amount of M. fermentans incognitus DNA also stained positively with M. fermentans incognitus-specific rabbit antiserum. Direct examination by electron microscopy of these tissues revealed M. fermentans incognitus particles. Clusters of M. fermentans incognitus particles could most frequently be found in the cytoplasm of hepatocytes and degenerating Kuffer cells. The nearly spherical particles were 140–280 nm in diameter, had well-defined outer limiting membranes and a densely packed granular or thin tubular internal structure. Occasionally, these M. fermentans incognitus particles were seen in the nuclei of cells with prominent pathological changes. Some M. fermentans incognitus particles were also noted in the extracellular tissue matrix. The necropsy tissues of liver and spleen obtained from the control monkey which did not contain M. fermentans incognitus DNA did not stain with M. fermentans incognitus-specific antiserum and did not have similar M. fermentans incognitus particles.

In an attempt to reisolate M. fermentans incognitus from M. fermentans incognitus-inoculated monkeys, the peripheral blood mononuclear cells obtained from the moribund monkeys were co-cultivated with normal human peripheral blood mononuclear cells (PBMC), NIH/3T3 cells and monkey BSC cells. Supernatants of the cultures were assayed for the presence of M. fermentans incognitus-specific antigens and DNA once every week. The cultures were maintained for three months without evidence of M. fermentans incognitus growth. All the cultures were also examined for the presence of reverse transcriptase enzyme activity representing growth of retroviruses. Homogenates of necropsy tissues such as liver and spleen were also inoculated into NIH/3T3 cells and monkey BSC cells. No M. fermentans incognitus was successfully recovered in any of these attempts.
EXAMPLE 11
Detection of Antibodies Against *M. fermentans* incognitus

Sera from AIDS patients and from normal subjects were analyzed by the immunoperoxidase staining procedure as described by Hsu et al., supra. Briefly, persistently infected Sb51 cells or normal NIH/3T3 cells were grown in low cell density on sterile glass slides. The culture slides were fixed in acetone at room temperature for five minutes. After washing in Tris-buffered saline (TBS), pH 7.6, 0.05M, the slides were first incubated with 1% normal horse serum containing 100 g/ml avidin (Sigma) for 30 minutes, and then incubated with saturated solution of biotin (Sigma) in TBS for an additional 15 minutes. This initial step has been shown to minimize any nonspecific reaction derived from avidin-biotin-peroxidase complex (ABC). The human antisera from AIDS patients or normal subjects were then used at 1:200 dilution followed by biotin-labelled goat anti-human immunoglobulin (Tago, Burlingame, Calif.) at 1:200 dilutions and ABC (Vector Lab., Burlingame, Calif.). Each incubation step was conducted for 30 minutes with extensive washing between steps. The color reaction was developed in DAB-NiH2O2 solution and counterstained with methyl green. Controls for the technique were performed by omitting the secondary antibody.

Sera of patients with AIDS produced positive immunochemical reactions with these infected cells, but not with normal NIH/3T3 cells (FIGS. 12(C) and 12(B), respectively). The reaction appeared to be positive in both nuclei and cytoplasm of Sb51 cells. However, many of the nuclei stained significantly stronger than the cytoplasm. A population of smaller round cells with apparently fewer cellular processes were found to be most heavily stained. Using this assay, 23 of 24 sera from AIDS patients, whether they presented with Kaposi's sarcoma, Kaposi's sarcoma with opportunistic infections, or opportunistic infections alone, were positive (Table 3). Serum from only one AIDS patient, with both Kaposi's sarcoma and opportunistic infections, showed weak positivity. Twenty-six of 30 non-AIDS normal human sera showed no reactivity to the infected Sb51 cells. One such negative reaction is shown in FIG. 12(A). The other four sera showed mild reactivity to these cells. However, staining intensity was significantly less than that seen in the reactions of AIDS patients' sera.

### Table 3

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Number Positive for Antibodies to Sb51 Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with AIDS</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
</tr>
<tr>
<td>Homosexual</td>
<td>1*</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
<tr>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
</tr>
<tr>
<td>Homosexual</td>
<td>8</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
</tr>
</tbody>
</table>

### Example 12
Identification of *M. fermentans* incognitus Infected Cells in Tissues of AIDS Patients

Lymph node, spleen, Kaposi's sarcoma and brain tissues from AIDS patients were fixed in Formalin and processed in paraffin sections. An immunoperoxidase assay, such as described in Example 11, was performed using antisera from mice or rabbits prepared as described in Example 8 in place of the antisera from AIDS patients. *M. fermentans* incognitus infected cells were identified in virtually all of the tissues examined. Electron microscopy was performed to confirm the infection by *M. fermentans* incognitus. Mature *M. fermentans* incognitus cells were also seen in some of the cells of the infected tissues.

### Example 13
Transmission of Cell-Free *M. fermentans* incognitus

Sb51 cells (about 2 × 10⁷ cells) were harvested following trypsinization. The cell pellet was resuspended in 2 ml of RPMI-1640 media with 10% sorbitol (w/v). The suspension was then subjected to five cycles of freezing and thawing followed by clarification of cell debris as described above. Supernatant containing *M. fermentans* incognitus was diluted in 20 ml of RPMI-1640 with 10% bovine calf serum and filtered through a 0.22 micron filter. The filtered supernatant was added to four 75-cm² tissue culture flasks containing 70% to 80% confluent normal NIH/3T3 cells, human embryo fibroblasts or monkey BSC cells (about 5 ml of filtered supernatant were added to each flask). The infected cultures were split one week later and replenished with fresh media. The cultures were kept for an additional week. At the end of two weeks, two flasks of cells were used for the next cycle of cell-free, *M. fermentans* incognitus transmission. The other two flasks were used for DNA extraction or antigen determination. Equal numbers of normal NIH/3T3 cells, instead of Sb51 cells, were cultured in parallel through each cycle of cell-free *M. fermentans* incognitus transmission as controls.

### Example 14
Molecular Cloning and Sequencing of *M. fermentans* incognitus

DNA was phenol extracted from sucrose-banded *M. fermentans* incognitus derived from Sb51 cells which were first lysed by 0.5% sodium dodecyl sulfate (SDS) and treated with proteinase K (200 mg/ml), for 1 hour at 60°C. Then 3 hours at 37°C. The alcohol precipitated DNA was treated with RNase. An EcoRI partial digest of the *M. fermentans* incognitus-enriched DNA was cloned into bacteriophage lambda charon 28. The lambda-conbinant clones were screened by differential
plaque hybridization, on duplicate sets of filters, with
32P-labeled DNA derived from gradient banded M.
incognitus versus that of normal NIH/3T3 cells. One
clone which had specifically hybridized to M. ferment-
tans incognitus DNA probe, but not to 3T3 DNA probe
was identified. The insert of the positive phase clone
was cloned into the EcoRI site of Bluescript KS
(M13) vector (Strategene). Two cloned probes, 8.6
kilobase (psb-8.6) and 2.2 kilobase (psb-2.2) were
obtained. The specificity of probes psb-8.6 and psb-2.2
was further verified by Southern blot analysis of DNA iso-
olated from M. fermentans incognitus and Sb51 cells
versus normal NIH/3T3 cells. To obtain sequence in-
formation, single-stranded DNA of clone psb-2.2 was
prepared by infection of the cells with helper phage
(Bluescript instruction manual, Strategene). About 200
base sequences starting from the EcoRI site at one end
of the insert fragment of psb-2.2 were obtained, using a
dideoxyxynucleotide sequencing method. The base se-
quence is set forth in SEQ ID NO:2.

EXAMPLE 15
Southern Blot Hybridization of M. fermentans
incognitus

Restriction endonuclease cleavage of M. fermentans
incognitus or cellular DNA was carried out with a 10-fold excess of enzymes under the conditions recom-
mended by the manufacturer (BRL).

The enzyme digests of DNA were subjected to gel
electrophoresis in 1% agarose and transferred onto
nitrocellulose membranes by the Southern blot method.
Each filter was prehybridized at 42°C for at least 4
hours in 50% formamide, 5x SSC (standard saline ci-
trate), 0.2% SDS, 20 mM Tris-HCl (pH 7.5), 2 mM
EDTA, 5x Denhart’s solution, and 350 microgram/ml
denatured salmon sperm DNA. Each filter was then
hybridized with 107 cpm of 32P-labeled probe (specific
activity after hybridization, the blots were washed at
60°C in 2x SSC, 0.5% SDS for 90 minutes with three
changes and then wrapped in sheets of saran wrap
and exposed to Kodak XAR film at −70°C with intensify-
ing screens for 2–20 hours depending upon the intensity
of the hybridized signals. For the reuse of the mem-
brane, the filters were boiled in 0.1x SSC, 0.1% SDS for
10 minutes to remove the previous M. fermentans incog-
nitus probe after autoradiographic exposure, and rehy-
bridized with 32P-labeled insert fragment of psb-8.6 as
previously described.

Use of the filters and results of such use are presented
in Example 19 below.

EXAMPLE 16
Analysis of Taq DNA Polymerase-Catalyzed PCR
Amplification Products

The amplification of selective DNA sequences by
Taq DNA polymerase chain reaction is known (U.S.
Pat. No. 4,683,202). Briefly, each 100 microliter reac-
tion mixture contained 1 microgram of human tissue
DNA in 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM
MgCl2, each primer (RS47 (SEQ ID NO:13) and RS49
(SEQ ID NO:14)) at 1 microM, each dNTP at 200 mi-
croM, gelatin at 100 micrograms/ml, and 2 units of Taq
DNA polymerase. The mixtures were heated at 94°C
for 2 minutes before the addition of DNA polymerase.
The samples were overlaid with 50 microliters of mini-
eral oil and subjected to 35 cycles of selective DNA
amplification. The thermal cycle was manually con-
ducted in three separate water baths as follows: 1 min-
ute at 52°C, 1 minute at 72°C, and 30 seconds at 94°
C. After the amplification, the reaction was stopped
by addition of EDTA (final concentration, 20 mM). Ten
microliter aliquots from each sample product were
removed and electrophoretically fractioned in a 6% poly-
acrylamide gel. The fractionated DNA was electropho-
ticated onto a Zeta membrane (Bio/Rad) at 40 volts for 90
minutes, followed by denaturation and fixation in 400
mM NaOH, 2 mM EDTA for 10 minutes at room tem-
perature. The Zeta membrane filter was rinsed three
times with 2x SSC in 20 mM Tris-HCl (pH 7.5), and air
dried for 10 minutes. Prehybridization of the blots was
carried out as previously described except the solution
contained 4x SSC and 1% SDS. A 22-base synthetic
oligonucleotide probe (RS48 (SEQ ID NO:1)), was 5’
end-labeled with 32P and hybridized to the filter at 30°
C. for 16 hours in the prehybridization solution contain-
ing 30% formamide. The blots were washed at 34° C. in
2x SSC, 0.5% SDS for 45 minutes with three changes
and at 37°C for an additional 2 minutes.

Use of the blots and the results of such use are pre-
seved below in Example 19.

The preferred PCR assay utilizes primers RW004 and
RW005 and probe RW006 as described in Example 5.

EXAMPLE 17
DNA and Antigen Dot-Blot Analysis of M. fermentans
incognitus

After 11 cycles of cell-free M. fermentans incognitus
transmission, the control and M. fermentans incognitus
infected NIH/3T3 cells of Example 13 were subjected to
M. fermentans incognitus isolation (see Example 7,
above). Ten microliter and/or twenty microliter sam-
ple from each fraction of the isopycnic sucrose gradi-
ent were first diluted to 400 microliters with PBS and
then dot-blotted onto nitrocellulose paper under vac-
uum. The dot was blocked with 5% non-fat milk and
reacted with pre-immunized or post-immunized rabbit
antiserum (1:400 in PBS) at 37° C. for 3 hours. The blot
was then developed with alkaline phosphatase conju-
gated goat anti-rabbit IgG (1:5000, in PBS) at 37° C. for
1.5 hours, followed by the addition of the substrates
Nitro Blue Tetrazolium (50 mg/ml in 70% dimethylfor-
mine) plus 5-Bromo-4 Chloro Indolyl phosphate (50
mg/ml) (Promega; Madison, Wis.). Between each of the
above steps, the blots were washed five times with PBS
and Tween 20 (1%), five minutes each wash. For ho-
mosologous DNA detection, the dotted blots were alka-
line treated, neutralized and probed with 32P-labeled
nick-translated psb51-8.6 or psb51-2.2 probes as previ-
ously described in Example 14.

Use of the blots and the results of such use are pre-
seved below in Example 19.

EXAMPLE 18
Immunohistochemistry for Detecting M. fermentans
incognitus Antigens in Infected Tissues

Deparaffinized sections were incubated with 10% Bovine
serum albumin (Sigma Chemical Co.) in Tris-
buffered saline (TBS, 0.05M Tris, pH. 7.4 saline) for 39
minutes, rinsed briefly with TBS, and covered with
rabbit antiserum from Example 12 (1:100 dilution). Slides
were refrigerated overnight. After returning to room
temperature, the slides were rinsed with 1% albumin in
TBS. Slides were then covered with secondary antisera.
Biotin-labelled horse anti-rabbit immunoglobulin (Vec-
tor Lab., Burlingame, Calif.) was added at a 1:200 dilution as the secondary antiserum, followed by the avidin biotinylated peroxidase complex (ABC) reagent (Vector Lab., Burlingame, Calif.). Each incubation step was conducted for 30 minutes with three TBS washes between steps. The color reaction was developed in Diaminobenzidine and H₂O₂ substrate and counterstained with hematoxylin.

Rabbit antiserum which reacted specifically with *M. fermentans* incognitus-Sb51 was used to stain formalin-fixed paraffin embedded lymph node and brain tissues of patients with AIDS. In the immunohistochemical study, reticuloendothelial cells or macrophages in the subcapsular sinus of a lymph node (Table 4, patient #1) were most often stained positively (FIG. 13). Brain from the autopsy of a patient with central nervous system symptoms and histopathologic evidence of subacute encephalitis without known etiology, contained many positively stained degenerating cells in lesions with diffuse infiltration of mononuclear lymphohistiocytes. Positive immunohistochemical reactivity was located in both nuclei and cytoplasm of swollen and disrupted cells. More peculiarly, brains from the autopsy of three other patients with CNS symptoms, but without histopathological evidence of encephalitis, also had numerous positively stained inclusion-like spherical structures (FIG. 14). The structures, most likely originating from neural cells with unique pathological changes, were inconsiderable in routine hematoxylin and eosin stained sections.

DNA from two of the three brains were available for PCR study and had positive *M. fermentans* incognitus DNA information after selective gene amplification (Table 4, patient #2 and #3). The positively stained structures were more common in periventricular and perivascular areas. Normal rabbit serum (Gibco Co.) and the rabbit serum obtained before immunization with *M. fermentans* incognitus did not stain these brains. Furthermore, the immunohistochemical reactivity of the rabbit antiserum with either Sb51 cells or purified *M. fermentans* incognitus, but not with normal NIH/3T3 cells or spontaneously transformed NIH/3T3 cells. Eleven autopsy brain tissue obtained from non-AIDS patient were used as controls. Brains from autopsies of patients with fatal rickettsial infection, bacterial sepsis, disseminated mycobacteriosis and CNS metastatic disease served as controls. No positive reaction was observed in these control non-AIDS tissues.

<table>
<thead>
<tr>
<th>TABLE 4-continued</th>
<th>Clinico-Pathological Profiles of Patients with AIDS and Analysis of Specific DNA Amplification</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>Clinical and/or Post Mortem Diagnosis</td>
<td>Tissue DNA for PCR</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>4.</td>
<td>37 y.o.w. male homosexual, PCP, and CMV infection and KS</td>
<td>1) Spleen</td>
</tr>
<tr>
<td>5.</td>
<td>45 y.o.w. male homosexual, KS, CMV and PCP infection</td>
<td>1) KS</td>
</tr>
<tr>
<td>6.</td>
<td>28 y.o.w. male homosexual with KS without IO</td>
<td>1) PBMC</td>
</tr>
<tr>
<td>7.</td>
<td>43 y.o.w. male homosexual with KS without IO</td>
<td>1) PBMC</td>
</tr>
<tr>
<td>8.</td>
<td>26 y.o.b. male homosexual with KS without IO</td>
<td>1) LN</td>
</tr>
<tr>
<td>9.</td>
<td>24 y.o.w. male homosexual with KS and myocarditis</td>
<td>1) Spleen</td>
</tr>
<tr>
<td>10.</td>
<td>31 y.o.w. male homosexual with KS, PCP, CMV and MAI infections</td>
<td>1) Spleen</td>
</tr>
<tr>
<td>11.</td>
<td>Diffuse histiocytic malignant lymphoma</td>
<td>1) Spleen</td>
</tr>
<tr>
<td>12.</td>
<td>Renal cell carcinoma</td>
<td>1) Liver</td>
</tr>
<tr>
<td>13.</td>
<td>Chronic active hepatitis</td>
<td>1) Brain</td>
</tr>
<tr>
<td>14.</td>
<td>Metastatic Ewing sarcoma in lung and liver</td>
<td>1) Ewing sarcoma</td>
</tr>
<tr>
<td>15.</td>
<td>Normal delivery placenta</td>
<td>1) Placenta</td>
</tr>
</tbody>
</table>

Labels ++ +, + + +, + and — denote high-level, intermediate level, low level and negative, respectively, for the relative intensities of the diagnostic band observed in the autoradiograms in FIG. 15.

GI - Opportunistic Infection
MAI - Mycobacterium Avium-Intracellular
KS - Kaposi's sarcoma
LN - Lymph node
PBMC - Peripheral Blood Mononuclear Cells
PCR - Polymerase-chain reaction

EXAMPLE 19

DNA Probes for the Direct Detection of *M. fermentans* incognitus DNA in Infected Tissues

DNA was extracted from the fractions of Example 6 and digested with EcoRI enzyme. Two molecular clones carrying 8.6 kb and 2.2 kb inserts, designated psb-8.6 and psb-2.2, were obtained. When used as probes, these clones specifically hybridized to DNA of Sb51 cells (lanes 1, 2) but not to that of parental NIH/3T3 cells (lanes 3, 4) (FIG. 15). These cloned probes were used to assay infectivity of *M. fermentans* incognitus in cell cultures. The gradient banded *M. fermentans* incognitus from Sb51 cells infected normal NIH/3T3 cells after being filtered through at 0.22 micron filter. The psb-8.6 probe specifically hybridized to DNA of NIH/3T3 cells which were harvested after
each round of cell-free *M. fermentans* incognitus trans-
mission (FIG. 16). Blotted filter containing 10 μg EcoRI
digested DNA from cells of sb8 (lane 1), original, nor-
mal NIH/3T3 cells (lane 2), 7th cycle cell-free VLIA
transmission control NIH/3T3 cells (lane 3), 11th cycle
cell-free VLIA transmission control NIH/3T3 cells
(lane 4), and 3rd cycle (lane 5), 5th cycle (lane 6), 7th
cycle (lane 7), 9th cycle (lane 8), and 11th cycle (lane 9)
of cell-free VLIA transmission in NIH/3T3 cells. Lane 10
contained DNA of partially purified VLIA. The blot
was probed with p32 labeled psb8-1-8.6. Similarly, the
psb-2 probe also specifically hybridized to DNA from
*M. fermentans* incognitus infected NIH/3T3 cells in
each cycle of passage, but not from control NIH/3T3
cells.

The 32P-labeled psb-8.6 probe was also used for de-
tection of *M. fermentans* incognitus in isopycnic sucrose
gradients which were originally designed to band
*M. fermentans* incognitus from Sb51 cells. *M. fermentans*
incognitus isolated after 11 cycles of cell-free passage
in NIH/3T3 cells had similar physical properties and was
concentrated in the fractions of density between 1.17
and 1.20 (gm/ml) (FIG. 17A). The parallel control
NIH/3T3 cultures following 11 cycles of cell-free trans-
mission did not contain *M. fermentans* incognitus. Im-
munochemical staining by rabbit antiserum raised
against *M. fermentans* incognitus originally isolated
from Sb51 cells also revealed that *M. fermentans*
incognitus was localized in these fractions (FIG. 18). FIG.
18A was stained using preimmunized rabbit serum and
FIG. 18B was stained with post-VLIA immunizations
rabbit antiserum. Gel electrophoretic analysis of the end-
labeled EcoRI or HindIII digests of the gradient-
banded *M. fermentans* incognitus indicated a minimum
molecular weight estimate for *M. fermentans* incognitus
of greater than 150,000 bp.

To determine whether there was any significant ho-
mology of *M. fermentans* incognitus to large human
DNA viral agents, Southern blot hybridizations were
performed with each filter containing the restriction
enzyme-treated DNA from purified *M. fermentans*
incognitus, NIH/3T3, and one of the following viral ge-
nomic DNA: HSV-2, VZV, EBV, CMV, HBLV, vac-
cinia pox virus and mouse CMV virus. Each filter was
hybridized to 32P-labeled corresponding viral DNA
probe, then washed and analyzed by autoradiography.
The incorporated viral probe was subsequently re-
moored by boiling the filters before rehybridization with
32P-labeled insert fragment of psb-8.6. No cross-hy-
bridization of *M. fermentans* incognitus probe psb-8.6
occurred to any of the human herpesviruses, vaccinia pox
virus or mouse CMV (FIG. 19). Southern blot hy-
bridization comparing VLIA DNA to DNA from known
human herpesviruses, vaccinia virus, MCMV, and
HVS. Blotted filters contained DNA of *VLIA* (A-H,
lanes 1, 2), normal NIH/3T3 (A-H, lanes 3, 4), HSV-2
(A, lanes 5, 6) VZV (B, lanes 5, 6), EBV (C, lanes 5, 6),
CMV (D, lanes 5, 6), HBLV (E, lanes 5, 6), vaccinia
virus (F, lanes 5, 6), MCMV (G, lanes 5, 6), and HVS
pT (H, lane 5). DNA in lanes 1, 3, and 5 were digested
by Eco R I: DNA in lanes 2, 4, and 6 were digested by
Bam HI. HVS pT 7.4 (H, lane 5) was digested with Taq
I. The p32 labeled probes for set I were HSV-1 pHSV-
106 (A), VZV pEco A (B), EBV pBam W (C), CMV
pCMH-35 (D), HBLV pZVH-70 (E), vaccinia pEh-
(F), MCMV pAMB-25 (G), and HVS pT 7.4 (H). Each
blot (A-H) of set I was boiled to remove incorporated
viral probe and then reprobed with p32 labeled insert
fragment of psb-8.6 (set II).

Conversely, while they hybridized to the homolo-
gous genomic DNA, one of the other viral probes hy-
bonded to the lanes containing *M. fermentans* incog-
nitus DNA digested with either EcoRI or BamHI.
pHSV-106 originated from HSV type I hybridized to
the genomic DNA of HSV type II, but not to the *M.
fermentans* incognitus DNA.

A viral probe of 7.4 kb DNA (pT 7.4) from Herpesvi-
rus saimiri (HVS) of squirrel monkeys was also exam-
inied. The viral probe did not hybridize to *M. fer-
mentans* incognitus DNA. In some of the rehybridized
filters, very weak bands resulting from incomplete removal of
the previously hybridized viral probes could be noted.
The weak signals served as useful reference points for
the newly appearing bands obtained after rehybridiza-
tion with psb-8.6 probe.

To investigate *M. fermentans* incognitus infection in
humans, the recently developed, a sensitive method of
selective DNA amplification, polymerase chain reac-
tion (PCR) (U.S. Pat. No. 4,683,202) was used. (As
discussed above in Example 5, it is preferred to use
RW004 (SEQ ID NO:15) and RW005 (SEQ ID NO:16)
as primers because a more sensitive assay is possible
with these primers.) One end of the *M. fermentans*
incognitus DNA in the psb-2.2 bluescript clone was se-
quenced. Primer pairs of synthetic oligonucleotides,
designated as RS47 (SEQ ID NO:13) and RS49 (SEQ
ID NO:14), with *M. fermentans* incognitus-specific se-
quences and Taq DNA polymerase were used for 35
reaction cycles of *M. fermentans* incognitus specific
DNA amplification. The positions of RS47 (SEQ ID
NO:13) and the complementary sequences of RS49
(SEQ ID NO:14) span the first 160 nucleotides of psb-
2.2 *M. fermentans* incognitus DNA (SEQ ID NO:2).
The amplified DNA carrying *M. fermentans* incognitus-
specific genetic information revealed positive signals,
when probed with 32P end-labeled synthetic oligonucle-
otide RS48 representing a segment of the intervening
sequences between RS47 (SEQ ID NO:13) and RS49
(SEQ ID NO:14).

Ten patients with AIDS have been examined and
were seropositive for HIV and had either typical oppor-
tunistic infections such as pneumocystis carinii pneu-
monia (PCP), toxoplasmosis, CMV infection or Kapos-
i's sarcoma (Table 4, subjects #1 to #10). Analysis of
the amplified DNA products revealed that a diagnostic
160 bp DNA fragment and a slower migrating frag-
ment(s) associated with a positive homologous signal,
were identified in samples derived from seven of the ten
AIDS patients tested. Representative results of nine
positive samples and two negative samples obtained
from seven AIDS patients are shown in FIG. 20. No
positive signal could be detected in any of the six DNA
samples derived from five control non-AIDS subjects
(Table 4, subjects #11 to #15). As summarized in Table
4, patient #1 had *M. fermentans* incognitus genetic
material in the lymph node, liver and spleen but not in
the brain. However, both patients #2 and #3 had posi-
tive *M. fermentans* incognitus-specific DNA products
in the brain samples.
EXAMPLE 20
Vaccine Containing Cells Infected by *M. ferments* incognitus

Sixteen chimpanzees are divided into four groups. Group A is inoculated intravenously with 1 ml of the novel *M. ferments* incognitus. Group B is inoculated with 1 ml of fluid containing 10⁶ *M. ferments* incognitus-infected NHI/3T3 cells. Group C is inoculated with 1 ml of fluid containing 10⁶ inactivated *M. ferments* incognitus-infected NIH/3T3 cells, and Group D is the control group and did not receive an inoculation.

All chimpanzees in Groups A and B developed symptoms of AIDS. However, none of the chimpanzees in Groups C and D developed the symptoms of AIDS. The chimpanzees of Group C are rendered immune to subsequent challenge of intravenous inoculation with 1 ml of *M. ferments* incognitus or 1 ml containing 10⁶ *M. ferments* incognitus-infected NIH/3T3 cells.

EXAMPLE 21
*M. ferments* incognitus Identified In Non-AIDS Patients

Six patients from six different geographic areas who presented with acute flu-like illnesses were studied. The patients developed persistent fevers, lymphadenopathy or diarrhea, pneumonia, and/or heart, liver, or adrenal failure. They all died in 1-7 weeks.

These patients had no serological evidence of HIV infection and could not be classified as AIDS patients according to CDC criteria. The clinical signs as well as laboratory and pathological studies of these patients suggested an active infectious process, although no etiological agent was found despite extensive infectious disease work-ups during their hospitalization.

Post-mortem examinations showed histopathological lesions of fulminant necrosis involving the lymph nodes, spleen, lungs, liver, adrenal glands, heart, and/or brain. No viral inclusion cells, bacteria, fungi, or parasites could be identified in these tissues using special tissue stains. However, the use of rabbit antiserum and the monoclonal antibodies raised against *M. ferments* incognitus (Example 8), the pathogen shown to cause fatal systemic infection in primates (Example 10), revealed *M. ferments* incognitus antigens in these necrotizing lesions. In situ hybridization using a ³⁵S labeled *M. ferments* incognitus-specific DNA probe (Example 18) also detected *M. ferments* incognitus genetic material in the areas of necrosis.

Furthermore, *M. ferments* incognitus particles were identified ultrastructurally in these histopathological lesions. *M. ferments* incognitus was associated with the systemic necrotizing lesions in these previously healthy non-AIDS patients with an acute fatal disease.

Typical areas of necrosis due to the *M. ferments* incognitus infection of these patients are shown in FIG. 21. Most of the tissues which had massive necrosis showed only minimal lymphocytic or histiocytic response and few neutrophils (FIGS. 21A, B and C). FIG. 21A is a photomicrograph of splenic tissue (x 30.5). FIG. 21B shows the peripheral margin of necrosis of 21A (x 153). FIG. 21C is a photomicrograph of lymph node tissue (x 15.25). Occasionally, a chronic or acute inflammatory reaction could be identified in the areas of necrosis (FIG. 21D), FIG. 21D is a photomicrograph of adrenal gland tissue (x 153).

Representative samples of the immunostained tissues of these patients are shown in FIGS. 22A-D. FIG. 22A is a photomicrograph of spleen tissue (x 80). FIG. 22B is a higher magnification of the margin of necrosis of 22A (x 353). FIG. 22C is a photomicrograph of lymph node tissue (x 257). FIG. 22D is a higher magnification of cells with positive cytoplasmic staining of 22C (x 706). FIG. 22E is a photomicrograph of hemorrhagic necrosis in adrenal gland tissue (x 706). The areas which displayed the highest concentration of *M. ferments* incognitus related antigens were often at the margin of necrosis. However, the necrotic center and peripheral unaffected areas had relatively low reactivity. Most of the positively stained cells were identified as lymphocytes or histiocytes in the lymph nodes and spleen, or reactive mononuclear cells in the liver, lungs, adrenal glands and heart.

Immunostaining of control tissues with necrotizing lesions from patients with cat scratch disease, Hodgkin's disease, malignant lymphoma, cryptococcal fungal infections and hemorrhagic necrotic tissues of Hairy cell leukemia did not display a positive reaction. Serum obtained from the same rabbit before immunization with *M. ferments* incognitus antigens also failed to display a positive immunoreaction in the necrotizing lesions of the six patients.

Using a ³⁵S radiolabeled psb-2.2 *M. ferments* incognitus DNA probe (Example 18), strong labeling of clusters of cells at the margins of necrosis of the affected tissues was observed. The affected tissues tested were formalin-fixed, paraffin-embedded spleen, lung, lymph node, adrenal gland liver and bone marrow. The intensity of the labeling, or the number of grains localized in the cells at the margin of necrosis was well above the level present at either the necrosis (FIGS. 23A and B). However, there were also clusters of apparently viable cells in the necrosis which were also strongly labeled (FIG. 23C). FIG. 23A shows strong labeling of cells at the peripheral zone of necrosis (x 76.5). FIG. 23B is a higher magnification of 23A (x 422). FIG. 23C shows the occasional positive labeling in an area of diffuse necrosis in the spleen (x 150). The inset of 23C is a higher magnification (x 422).

Formalin-fixed, paraffin-embedded liver and spleen tissues from a patient with pancreatic carcinoma were used as negative controls, and showed no labeling above background levels. A control probe of ³⁵S labeled cloning vector DNA, not containing psb-2.2 *M. ferments* incognitus DNA did not label any of the tested tissues (FIG. 23D). FIG. 23D is the same area of FIG. 23C in the consecutive tissue section, hybridized with ³⁵S labeled cloning vector DNA not containing psb-2.2 *M. ferments* incognitus DNA (x 150) (i.e., control for 23C).

Areas of the necrotizing lesions which immunostained most positively for *M. ferments* incognitus specific antigens were examined by electronmicroscopy. Particles with characteristic ultrastructural features of *M. ferments* incognitus were directly identified in all the lesions. These particles in the areas of necrosis, morphologically resembled *M. ferments* incognitus previously identified in Sb51 cells (Example 4) and in the tissues of experimentally inoculated monkeys (Example 10). The particles were heterogeneous in size and shape, with most particles being spherical and about 140 to 280 nm in diameter. At the margin of necrosis, the *M. ferments* incognitus particles were located in the cytoplasm of cells with apparently no cytopathic changes, or in fragments of cytoplasm from completely disrupted...
cells (FIG. 24). FIG. 24 shows electron micrographs of tissues derived from areas highly positive for M. fermentans incognitus-specific antigens. FIG. 24A is an electron micrograph at a margin of necrosis in adrenal gland tissues (Bar=1,000 nm). FIG. 24A2 is a higher magnification of 24A (Bar=100 nm). FIGS. 24B1, and 24B2 are electron micrographs of the peripheral zone of necrosis in lymph node tissue (Bar=1,000 nm). FIG. 24B2 is a higher magnification of 24B2 (Bar=100 nm).

Table 5, below, summarizes the profiles and histopathological findings for each of the six patients.

<table>
<thead>
<tr>
<th>Patient Profile</th>
<th>Salient clinical presentation</th>
<th>Duration of illness (weeks)</th>
<th>Tissue with necrotic lesions identified by biopsy or at autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 29-year old man</td>
<td>arthralgia, myalgia, conjunctivitis, persistent fever, hypercalcemia, liver failure (late), ARDS* (late)</td>
<td>4.5</td>
<td>spleen, lung</td>
</tr>
<tr>
<td>2 33-year old white woman</td>
<td>persistent fever, diarrhea, generalized lymphadenopathy, abnormal liver functions, seizure (late)</td>
<td>7</td>
<td>lymph nodes, liver, spleen, kidneys</td>
</tr>
<tr>
<td>3 40-year old white man</td>
<td>arthralgia, myalgia, sore throat, chest pain, persistent fever, malaise, diarrhea, finger numbness, comatose (late)</td>
<td>3.5</td>
<td>adrenal glands (bilateral), heart, brain</td>
</tr>
<tr>
<td>4 31-year old black woman</td>
<td>vomiting and diarrhea, tremor, fever, epigastric and chest pain, abnormal liver functions, headache</td>
<td>1.5</td>
<td>liver, spleen</td>
</tr>
<tr>
<td>5 23-year old white man</td>
<td>Watery diarrhea, vomiting, jaundice, arthralgia, myalgia, fever, malaise, nausea and vomiting, myalgia and weakness, liver failure and jaundice, confusion and hallucinations (late)</td>
<td>3</td>
<td>liver, heart</td>
</tr>
<tr>
<td>6 33-year old black man</td>
<td></td>
<td>1</td>
<td>spleen, liver</td>
</tr>
</tbody>
</table>

*ARDS - Adult Respiratory Distress Syndrome

EXAMPLE 22

Biochemical Properties and Characteristics of M. fermentans incognitus

In order to identify biochemical properties and characteristics of M. fermentans incognitus, a variety of analyses were performed on this pathogen. The analyses of biochemical properties, antigenic specificity, DNA homology and restriction pattern analysis show that M. fermentans incognitus is distinct from all other know species of human mycoplasma, but appears to be biologically, serologically and molecularly different from the recently described human mycoplasma. Mycoplasma genitalium, only closely related to M. fermentans, a rarely isolated human mycoplasma.

M. fermentans incognitus from culture supernatant of Sb51 cells (Example 4) was cultured in cell-free conditions using a modified SP-4 medium. SP-4 broth was prepared according to previously described procedures (Whitcomb, R. F., Methods in Mycoplasmology, Vol. I, Academic Press, Inc. pp. 147–158 (1983) and Tully, J. G. et al., Science 195, 892 (1977)), and then supplemented with 20% heat inactivated fetal bovine serum (FBS) (M.A. Bioproducts Cat. #14-901B, Lot No. 8M0320 for hybridoma). Modified SP-4 broth medium was further supplemented with 0.15 mg/ml niacin (nicotinic acid, Sigma), 0.15 mg/ml riboflavin (Sigma) 0.15 mg/ml L-arginine and 0.01 mg/ml nicotinamide adenine dinucleotide (NADH, Pharmacia). Modified SP-4 agar medium containing 1% Noble agar (Gibco) was dispensed into sterile plastic petri-plates (Falcon).

The cell debris from the Sb51 cells was first removed from 5 day-old culture supernatant by centrifugation at 1,500 rpm for 15 minutes. The supernatant was then pelleted in Sorvall superspeed centrifugation 10,000 rpm for 20 minutes. The particles pelleted from 50 ml of culture supernatant were resuspended in 1 ml of modified SP-4 medium and used as inoculum. The M. fermentans incognitus-containing suspension was 1:10 serially diluted with SP-4 medium and then inoculated (0.2 ml) into modified SP-4 broth culture medium (2 ml).

Culture incubation and observation

All broth cultures and agar media plates were either incubated at 37º C. or 30º C. in anaerobic Gas Pak jars (BBL, Microbiology Systems, Cockeysville, Md.), candle jars or in a regular incubator. The broth media were examined daily for three weeks. Broth cultures were observed macroscopically against a white background to facilitate detection of color changes. Positive broth cultures were confirmed by subculturing 0.1 ml volumes to fresh modified SP-4 both and agar plates as soon as any color change was detected.

The surface of the agar plates was scanned with the use of a low-power objective (X4) from a standard light microscope or an inverted microscope. Positive cultures were identified by characteristic colony morphology.

For the studies of antigenic and DNA analysis, M. hyorhinis 9ATCC #17981), M. orale (ATCC #23714), M. pneumonia (ATCC #15531), M. hominis (ATCC #15488), M. genitalium (ATCC #33530), M. salvarium (ATCC #23064), M. fermentans incognitus and Acholeplasma laidlawii (ATCC #23206) strains were cultured in modified SP-4 broth. U. urealyticum (ATCC #27618) was cultured in modified SP-4 broth supplemented with 0.03% urea.
The broth cultures appeared slightly turbid and an acidic shift in pH occurred after 10 to 14 days of incubation either at 30°C or 37°C. Cells grew slightly better in a candle jar than in aerobic conditions; observation of a pH shift usually occurred about one day earlier.

M. fermentans incognitus could be filtered through a 220 nm membrane filter and continued to grow in the broth filtrate. The cells grown in the modified SP-4 broth were examined by electron microscopy after either ultrathin sectioning or direct negative staining. Clusters of cell wall-free microorganisms which were bound by a single triple layered membrane, showed typical pleomorphic morphology of Mollicutes.

Most of the particles were spheroidal, but filamentous forms with occasional branching configuration, were also observed (FIG. 1A). In general, the average size of spherical M. fermentans incognitus particles in the broth cultures appeared to be much smaller than that of M. fermentans (180 nm versus 460 nm).

M. fermentans incognitus could also produce colonies on 1% Noble agar plates prepared from modified SP-4 media. Compared with some other human mycoplasmas, M. fermentans incognitus grew rather slowly and formed only small colonies (FIG. 1C). For comparison, colonies with a regular size and sharp edge formed by M. fermentans incognitus growing in a parallel modified SP-4 medium agar plate after a shorter incubation period are shown in FIG. 1D. The small colonies of M. fermentans incognitus became microscopically visible after 10 to 14 days of incubation. Most of the colonies were somewhat diffuse and irregular, and much of their growth occurred within the agar. However, under an inverted phase microscope, the small central area of the colony was found to grow even deeper into the agar and exhibited the appearance of a “fried egg” (FIG. 1C).

A single typical colony of M. fermentans incognitus was picked three times from consecutive agar plates. The cloned agent was then continuously grown and passed in the broth of modified SP-4 medium. There was no evidence of cell wall growth or conversion into a bacterium, when M. fermentans incognitus was cultured and passed in an antibiotic-free medium.

In order to verify the definite relationship between M. fermentans incognitus and what was previously identified as VLIA from Sb51 cells (prior patent application Ser. No. 265,920, filed Nov. 2, 1988), DNA from this cloned M. fermentans incognitus was isolated and compared with that of Sb51 cells containing VLIA. The DNAs were first digested with EcoRI, HindIII and PstI restriction enzymes. In the analysis of a Southern blot probed with either psb-8.6 or psb-2.2, DNA of M. fermentans incognitus grown in a cell free condition using modified SP-4 medium was identical to DNA of VLIA in Sb51 cells (FIG. 25). This tetrally cloned M. fermentans incognitus was later used for all the following assays in this study.

FIG. 25 shows analysis and comparison of DNA restriction patterns of VLIA and M. fermentans incognitus. Blot (A) and blot (B) were probed with 32P nick translated inserts of psb-8.6 and psb-2.2, respectively. Each lane in the gel contained 1 microgram of DNA from Sb51 cells infected with VLIA (lanes 1, 2, 3) and control NIH/3T3 cells (lanes 4, 5, 6) or 1 nanogram of DNA from M. fermentans incognitus cultured in modified SP-4 broth (lanes 7, 8, 9). DNA was predigested with restriction enzymes EcoRI (lanes 1, 4, 7) HindIII (lanes 2, 5, 8) and PstI (lanes 3, 6, 9). Arrows indicated the positions of standard size maker 23, 9.4, 6.7, 4.4, 2.3, and 2.0 kbp, respectively.

Biochemical characterization

The tests of glucose breakdown by oxidation or fermentation, and hydrolysis of arginine or urea were performed according to standard bacteriological techniques for the characterization of mycoplasma species (Alvotto, B. B. et al., Intl. J. Systematic Bacteriology 20, 35 (1970)). Specifically, glucose, arginine and urea media were prepared by adding 10 ml of 10% (w/v) test substrate and 1 ml of 0.5% (w/v) phenol red to 74 ml of modified SP-4 broth without glucose. Each medium was adjusted using 5N HCl or 4N NaOH to the following initial pH values: glucose medium, 7.6; arginine medium, 7.0; and urea medium, 7.0. Each broth medium was filtered through a 0.22 micrometer filter and dispensed in 5 ml amounts into screw-capped tubes.

All inoculated cultures were incubated at 37°C. Anaerobic cultures were kept in Gas Pak jars (Gibco) and candle jars. Tests were read daily. A drop of 0.5 pH unit or more in the glucose tube compared with the appropriate substrate control tube constituted a positive reaction; a rise of 0.5 pH unit or more in the arginine or urea tubes compared with the appropriate substrate control tubes constituted a positive test. The pH values were read by comparison with a set of standards ranging from pH 5.6 to 8.4. Positive and negative test control organisms were:

A) Glucose breakdown (both aerobic catabolism and fermentation)

Positive: M. fermentans and M. hyorhinis

Negative: M. orale

B) Arginine hydrolysis:

Positive: M. fermentans and M. orale

Negative: M. hyorhinis

C) Urea hydrolysis:

Positive: Ureaplasma urealyticum

Negative: M. fermentans

In comparison with other known species of human mycoplasmas, including M. pneumoniae and M. fermentans incognitus, M. fermentans incognitus appeared to be more fastidious in cultivation and did not grow in the conventional mycoplasma media (Table 5, presented at the end of this Example). Modified SP-4 (with the further addition of NADH, niacin and riboflavin) was the only medium able to support a continuous growth of M. fermentans incognitus. Serum was a necessary supplement which could not be replaced by albumin. Increased fetal bovine serum concentrations (at least up to 10 to 15% of supplement) in the modified SP-4 medium produced a growth response.

M. fermentans incognitus catabolized glucose under both aerobic and anaerobic conditions of cultivation (Table 6). M. fermentans incognitus hydrolyzed arginine and produced an alkaline shift in pH, albeit slower than M. fermentans incognitus. A prominent alkaline shift in pH occurred after an initial brief acidic shift in the M. fermentans incognitus broth culture. M. fermentans incognitus could not hydrolyze urea in the biochemical assay. The usual biological characteristics of this microorganism are apparently distinct from all the other human species but similar to M. fermentans, an other glycolytic and arginine-metabolizing mycoplasma (Kenny, G. E., Manual of Clinical Microbiology, American Society for Microbiology, Washington, D.C 4th Ed., pp.d 147-158 (1985)).
TABLE 6

Comparison of Growth and Biochemical Properties of Mycoplasma
incognitus to Eight Other Mollicutes

<table>
<thead>
<tr>
<th>Species</th>
<th>AL</th>
<th>MA</th>
<th>MHO</th>
<th>MHY</th>
<th>MP</th>
<th>MO</th>
<th>UU</th>
<th>MF</th>
<th>MI</th>
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<tbody>
<tr>
<td>(I) Ability of Growth in Different Culture Media*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayflick</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Brain &amp; Heart Infusion Broth</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>±</td>
</tr>
<tr>
<td>Mycotrim-TC</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Heart Infusion Broth</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Arginine Broth</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Boston Broth</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<td>+</td>
</tr>
<tr>
<td>A7 Agar</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SP-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Modified SP-4 (aerobic and candle jar)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

(II) Biochemical Properties:

<table>
<thead>
<tr>
<th>Glucose Breakdown</th>
<th>Oxidation (aerobic culture)</th>
<th>Fermentation (anaerobic culture)</th>
<th>Arginine Hydrolysis</th>
<th>Urea Hydrolysis</th>
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<tbody>
<tr>
<td>ND</td>
<td>-</td>
<td>ND</td>
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<td>-</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
</tr>
</tbody>
</table>

*All the culture media were supplemented with 20% fetal bovine serum.
*The SP-4 medium was supplemented with urea.


Southern blot DNA analysis

Restriction endonuclease cleavage and Southern blot hybridization using nick translated psb-8.6 nd psb-2.2 probes as well as 32P end-labeled RS48 were described previously (Examples 13-17). A cDNA probe of E. coli r-DNA (23S and 16S r-RNA, Pharmacia Cat. #27-2508-01) was prepared with 32P alphadexyadeno-
sine triphosphate by random primer extension method (Feinberg, A. P. et al., Anal. Biochem. 132, 6 (1983)) using cloned Moloney murine leukemia virus reverse transcriptase (from BRL) and random primer (Pharmacia) under the conditions recommended by the manufac-
ture of BRL. Two tenth micrograms of purified DNA isolated from cultures of each species of myco-
plasma were applied to each lane for gel electrophoresis after restriction enzyme digestion.

Molecular cloning of M. fermentans incognitus DNA

DNA was phenol extracted from a pure culture of M. fermentans incognitus grown in modified SP-4 medium. The alcohol precipitated DNA was treated with RNase.

A HindIII digest of the M. fermentans incognitus DNA was cloned into M13 mp18 Vector (Norrander, J. et al., Gene 26, 101 (1983)). The M13 mp18 recombinant clones were screened by plaque hybridization, on nitro-
cellulose filters, with 32P-labeled DNA derived from M. fermentans incognitus. One clone which had specifically hybridized to M. fermentans incognitus DNA probe was identified. The insert of 3.3 kilobase M. fermentans incognitus DNA (M1-H 3.3) was identified in the cloned probe. The cloned probe M1-H 3.3 used for Southern blot DNA analysis, had been radiolabeled with 32P alphadexyadeno-
merase I (USBC Co.).

Development and isotyping of monoclonal antibodies

Balb/c mice were immunized with heat inactivated (60° C. for 20 minutes) M. fermentans incognitus in complete Freund's adjuvant through the interperi-
toneal route. The mice were subsequently boosted twice at biweekly intervals, three weeks after the initial injection, with heat inactivated M. fermentans incognitus material in incomplete Freund's adjuvant. Four days after the last boost, the spleen was removed and the spleen cells were fused with NSI myeloma cells using polyethylene glycol as described in Galfre and Milstein (Methods of Enzymology Vol. 73, Academic Press, Inc., pp. 3-46 (1981)). The fused cells were then added to 96-well microtiter plates in hypoxanthine, aminopterin and thymidine supplemented medium to eliminate unfused myeloma cells. Culture supernatants in each well were then tested for the production of antibody by using M. fermentans incognitus antigen-
coated microtiter plates in an ELISA system.

Selected hybridomas were cloned by the limiting dilution assay in 96-well microtiter plates. Supernatants from wells demonstrating active growth were re-tested for antibody activity in the ELISA system. The speci-
ficity of the monoclonal antibodies was further crossed-
checked by using M. fermentans incognitus, Sb51 and NIH/3T3 cell antigen-coated microtiter plates. The generation of ascites fluid was accomplished by injecting ten million hybridoma cells into the peritoneal cavity of Balb/c Nu/Nu mice which had been primed with 0.5 ml of pristane, 5-7 days earlier. Ascites were harvested by inserting a 20 gauge needle and withdrawing the fluid. The material was clarified by centrifugation at 2500 rpm (300x g) for 10 minutes, and stored at -70° C. Isotyping was done using reagents from isotyping kit (Screeentype, Boehringer Mannheim Biochemicals) and Bio-Dot apparatus (Bio-Rad).

Analysis of genomic DNA by restriction enzyme mapping and comparison of specific sequence homol-
ogy were extremely useful in comparing different spe-
ices of mycoplasma. Ten different species of myco-
plasma, M. orale, M. hyorhinis, M. pneumonia, M. ar-
ginini, M. hominis, M. fermentans, M. genitalium, M. salivarium, U. urealyticum and A. laidlawii were obtained from ATCC and cultured in the modified SP-4 broth medium with or without specific supplement. DNA isolated from M. fermentans incognitus and these mycoplasmas was analyzed on Southern blots and probed with 32P labeled cloned M. fermentans incognitus DNA (psb-8, psb-2.2) or synthetic oligonucleotide (RS48).

FIG. 3 shows a comparison of DNA homology and restriction patterns between M. fermentans incognitus and other human mycoplasmas. The blots were probed with 32P-labeled, psb-8.6 (A), psb-2.2 (B), 32P end-labeled RS48 (C), 32P labeled MI-H 3.3 (D) and 32P labeled cDNA probe of E. coli ribosomal RNA (E). Each lane contained 0.2 microgram of EcoRI enzyme pre-digested DNA from Acholeplasma laidlawii (lane 1), M. arginiini (lane 2), M. hominis (lane 3), M. hyorhinis (lane 4), M. pneumoniae (lane 5), M. orale (lane 6), M. fermentans incognitus (lane 7) and M. fermentans incognitus (lane 8). Arrows indicate the positions of standard size marker 23, 9.4, 6.7, 4.4, 2.3, and 2.0 kbp, respectively.

One additional molecular clone, carrying the 3.3 kilobase insert of M. fermentans incognitus DNA, designated MI-H 3.3, was also used as a probe in the study. Although some homology with psb-2.2 was observed in the M. orale genome (FIG. 3B), no homology with RS48 (SEQ ID NO:1), the specific DNA sequences occurring at one terminal end of psb-2.2, and no homology with psb-8.6 or MI-H 3.3 could be identified in the M. orale genome.

However, DNA homology with psb-8.6, psb-2.2, RS48 and MI-H 3.3 were all found in the M. fermentans genome (FIG. 3A, B, C, D), but, the restriction patterns revealed by these probes were different between M. fermentans and M. fermentans incognitus. No similar DNA homology could be found in any other species of mycoplasma.

There is significant homology between the ribosomal RNA (r-RNA) genes of procaryotic mycoplasmas and those of Escherichia coli bacterium (Gaobel, U. B. et al., Science 226, 1211 (1984)). The same blot which had been probed consequentily with RS48 and MI-H 3.3, was reprobed with 32P labeled cDNA of E. coli r-RNA, after removing the previously incorporated probes by boiling the filter. This analysis of r-RNA genes revealed both a difference in numbers and size of the hybridization bands with each different species of mycoplasma tested (FIG. 3E). The characteristic restriction enzyme mappings of r-RNA genes in these Mollicutes enable the identification of related species. The EcoRI restriction pattern of r-RNA genes of M. fermentans incognitus and M. fermentans appeared to be identical (FIG. 3E) and was different from any other mycoplasma tested.

Antigenic analysis using polyclonal and monoclonal antibodies

The microorganisms harvested from each culture were washed once in phosphate buffered saline (PBS) and then resuspended in PBS. Protein concentrations of each suspension were determined using the Bio-Rad protein assay kit (Bio-Rad instruction manual). Antigen analysis with polyclonal and monoclonal antibodies was done using the Bio-Dot microfiltration apparatus (Bio-Rad).

One hundred microliter samples from each dilution which contained decremental amounts (either 1:4 or 1:10 dilution in PBS) of proteins were dot-blotted onto nitrocellulose paper under vacuum. The blots were blocked with 5% non-fat milk and reacted with either M. fermentans incognitus specific rabbit antiserum (1:1000 in PBS) (Lo, S-C et al., Am. J. Trop. Med Hyg. 40, 215 (1989)), or M. fermentans incognitus specific mule antiserum (1:4000 in PBS), provided by Dr. Richard A. Dol Guidice of Frederick, Md. The titers of the rabbit M. fermentans incognitus antiserum and the mule M. fermentans incognitus antiserum had previously been determined to be 20,000 and 80,000, respectively.

The blots were then reacted with biotinylated goat anti-rabbit IgG (Vector) and biotinylated goat antihorse IgG (Vector), respectively. In the antigenic analysis using monoclonal antibodies, the concentration of primary antibody was adjusted to 20 fold of each monoclonal antibody titer. The titers of these monoclonal antibodies were previously determined to be D81E7, 5.1x10^4; C69H3, 2.6x10^4; F89H7, 2.0x10^5; B109H8, 2.6x10^4; F11C6, 6.4x10^3; and C24H10, 2.6x10^4. The biotinylated horse antimouse IgG or goat antimouse IgM (Vector) were used as the secondary antibodies according to the specific isotype of each monoclonal antibody. Each incubating step was conducted for 30 minutes at room temperature with three Tris buffered saline-Tween 20 (0.2%) washes between steps. The color reaction was developed in diaminobenzidine and H2O2 substrate after formation of avidin-biotin complex.

Both biological characterization and DNA homology analysis indicated that M. fermentans incognitus was distinct from all other species of human mycoplasmas, but closely related to M. fermentans incognitus. Therefore, a detailed comparison between these two species was performed by studying their specific antigenicity.

Polyclonal rabbit antiserum raised originally against VL1a-Sh51 (Lo, S-C et al., Am. J. Trop. Med. Hyg. 40, 339 (1989)) was found to react with M. fermentans in addition to M. fermentans incognitus, but not with any other mycoplasmas examined (FIG. 2A). However, a larger amount of M. fermentans protein (>0.63 mg) was required to elicit the positive immunochemical reaction in this assay. The positivity of reaction quickly disappeared when the M. fermentans proteins were further diluted. In comparison, a 250-fold to 1000-fold lower concentration of M. fermentans incognitus proteins still carried a sufficient amount of antigenic determinants and exhibited positive reactions in the assay (FIG. 2A).

In the parallel assay, antiserum raised specifically against M. fermentans also reacted intensely with M. fermentans incognitus (FIG. 2B). The M. fermentans-specific antiserum appeared to cross react with A. laidlawii and M. orale when high concentrations (10 mg) of mycoplasma proteins were dot-blotted. M. fermentans antiserum reacted with the antigens of M. fermentans incognitus proteins. Both M. fermentans incognitus and M. fermentans proteins could be diluted to 40 ng per well and still elicit a positive reaction (FIG. 2B).

FIG. 2 shows antigenic comparison of M. fermentans incognitus, M. fermentans and other human mycoplasmas in immunoblots. Upper blot (A) was immunostained with rabbit antiserum raised specifically against M. fermentans incognitus. Lower blot (B) was immunostained with rabbit antiserum raised specifically against M. fermentans. The concentration of mycoplasma protein was dot-blotted decrementally (1:4 dilu-
tion) from lane 1 (10 mg) to lane 12 (2.5 pg). Row A (M. arginini), row B (A. laidlawii), row C (M. fermentans), row D M. hominis, row E (M. orale), row F (M. hyorhinis), row G (M. pneumoniae), row H (M. fermentans incognitus). In FIG. 2 C row A, B, C, D and F were immunostained with monoclonal antibodies D81E7, C69H3, F89H7, B109H8, F11C6 and C42H10, respectively. The concentration of mycoplasma protein was dot-blotted decrementally (1:10 dilution) from lane 1 (10 ug) to lane 8 (1 pg). Row a (M. fermentans incognitus) and Row b (M. fermentans).

In order to examine the possibility suggested by the above results that M. fermentans incognitus carried additional unique antigens which are not present in M. fermentans, a battery of monoclonal antibodies raised specifically against M. fermentans incognitus were prepared. All six M. fermentans incognitus monoclonal antibodies obtained, many with different isotypes, were found to react only with M. fermentans incognitus but not with M. fermentans (FIG. 2C). These monoclonal antibodies also did not react with any of the other nine Mollicutes examined.

Table 7 summarizes the results of the antigenic analysis using both polyclonal and monoclonal antibodies. The results confirmed that M. fermentans incognitus carries additional specific antigens which could not be identified in M. fermentans.

**TABLE 7**

<table>
<thead>
<tr>
<th>ANTIBODIES</th>
<th>ISOTYPE</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit antiserum Against MI</td>
<td>Polyclonal</td>
<td>M. arginini, A. laidlawii, M. fermentans</td>
</tr>
<tr>
<td>Mule antiserum against MF</td>
<td>Polyclonal</td>
<td>M. hominis, M. pneumoniae, M. orale, M. hyorhinis</td>
</tr>
<tr>
<td>D81E7</td>
<td>Monoclonal IgM/K</td>
<td>M. arginini, A. laidlawii, M. fermentans</td>
</tr>
<tr>
<td>C69H3</td>
<td>Monoclonal IgM/K</td>
<td>M. hominis, M. pneumoniae, M. orale, M. hyorhinis</td>
</tr>
<tr>
<td>F89H7</td>
<td>Monoclonal IgM/K</td>
<td>M. arginini, A. laidlawii, M. fermentans</td>
</tr>
<tr>
<td>B109H8</td>
<td>Monoclonal IgG3/K</td>
<td>M. hominis, M. pneumoniae, M. orale, M. hyorhinis</td>
</tr>
<tr>
<td>F11C6</td>
<td>Monoclonal IgG3/K</td>
<td>M. arginini, A. laidlawii, M. fermentans</td>
</tr>
<tr>
<td>C42H10</td>
<td>Monoclonal IgG1/K</td>
<td>M. arginini, A. laidlawii, M. fermentans</td>
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Labels + + +, +, ± and - denote the relative positivity of immunostaining results in FIG. 4. MA: M. arginini, AL: A. laidlawii, MF: M. fermentans, MHO: M. hominis, MO: M. orale, MHY: M. hyorhinis, MP: M. pneumoniae, MI: M. incognitus.

Direct immunofluorescence examination

Monoclonal antibody was purified from ascites fluid by high-salt precipitation and gel chromatography using Sephacryl-200 (Campbell, D. H. et al., *Method in Immunology* 2d Ed., W. A. Benjamin, Inc., p. 198 (1970)). Labeling of the purified antibody with fluorescein isothiocyanate (FITC) was done using the method described by Rinderknecht (*Nature* 193, 167 (1962)). The broth culture suspensions were directly smeared on the slides. The slides were air dried, fixed in 70% acetone, 30% methanol and stored at 4°C. The slides were directly immunostained with FITC conjugated monoclonal antibody and examined under a fluorescent microscope.

In this study of direct immunofluorescence staining, the FITC probe conjugated to the purified M. fermentans incognitus monoclonal antibodies which again revealed positive staining only in M. fermentans incognitus, but not in the other seven species of human mycoplasmas, including M. fermentans (FIG. 4).

FIG. 4 shows direct immunofluorescence staining of M. fermentans incognitus (A) and M. fermentans (B) using FITC conjugated monoclonal antibody D81E7 (X900).

**EXAMPLE 23**

Identification of *M. fermentans* incognitus Infection in Patients with Aids

Monoclonal antibodies developed against antigens from a pure culture of M. fermentans incognitus grown in modified SP-4 medium were used to immunohistologically identify *M. fermentans* incognitus infection in tissues of thymus, liver, spleen, lymph node or brain from 26 out of 32 patients with AIDS. *M. fermentans* incognitus infection was also identified in 2 placentas delivered by 2 patients with AIDS. The 32 patients tested were homosexuals, intravenous drug abusers or pediatric patients who had received transfusions.

*M. fermentans* incognitus specific DNA was also identified in the subject tissues using a 35S labeled psb-22 DNA probe and in situ hybridization. Although *M. fermentans* incognitus was found to be both cytotoxic and cytotoxic, the cellular immune response and inflammatory reaction to M. incognitus infection was often atypical.

**Patient profiles**

All 34 AIDS patients were selected according to CDC criteria (*JAMA* 258, 1143 (1987)). All patients were seropositive for HIV-related antigens. Unless specified below, all the patients belonged to the homosexual high risk group.

Four thymic tissues, 10 livers from patients with unexplained abnormal liver function tests, 8 spleens and 8 brains from patients with clinical CNS symptoms obtained at autopsy as well as 2 biopsied lymph nodes were used. Histopathological studies using special tissue stains did not reveal any bacterial, fungal or viral infectious agent in these tissues. All tissues obtained at autopsy and biopsy were previously fixed in 10% buffered formalin and embedded in paraffin blocks. Tissues of non-AIDS control subjects were also obtained from 10% formalin fixed and paraffin embedded blocks of autopsy tissues.
Immunohistochemistry and in situ hybridization

Deparaffinized and frozen section slides were incubated with 10% bovine serum albumin (Sigma Chemical Co.) in phosphate-buffered saline (PBS, Gibco Co., pH 7.4 saline) for 30 minutes, rinsed briefly with PBS, and covered with monoclonal antibodies (1:600 dilution).

Slides were kept refrigerated overnight. After returning to room temperature, the slides were rinsed with 1% albumin in PBS. The slides were then covered with secondary antibodies. Biotinylated horse anti-mouse IgG (H&L) or biotinylated goat anti-mouse IgM (H&L) (Vector Lab., Burlingame, Calif.) was added at 1:200 dilution in PBS as secondary antiserum and followed by the avidin biotinylated peroxidase complex (ABC) reagent (Vector Lab, Burlingame, Calif.). Each incubation step was conducted for 1 hour with extensive washing between steps. The color reaction was developed in DAB-H2O2 substrate and counterstained with hematoxylin.

Development of M. fermentans cognitans-specific monoclonal antibodies (C42H10, and D81E7) has been described previously in Example 21. In parallel, non-specific mouse monoclonal antibodies (IgM, MOPC 104E and IgG2aK, MOPC 141, Sigma) or monoclonal antibody (ascites) raised specifically against herpes virus (IgG1, MCA 255, clone R1, Bioproducts) were used as the primary antibodies and served as negative controls in immunohistochemistry. Detailed procedures of preparation of 35S radiolabeled psb-2.2 probe and in situ hybridization on formalin-fixed and paraffin embedded tissues were also described previously (Lo, S-C et al., Am. J. Trop. Med. Hyg. 41, 380 (1989)).

Electron microscopy

To retrieve formalin-fixed paraffin-embedded tissues for ultrastructural examination, immunohistochemistry positive areas of tissue sections on glass slides were circled. These exact area were then matched and identified on each individual paraffin block. Tissues of 1 to 2 mm in diameter were punched out from the blocks and deparaffinized in xylene. Processing of these tissues for E. M. studies were previously described in detail (Lo, S-C et al., Am. J. Trop. Med. Hyg. 41, 380 (1989)).

RESULTS

Thymus

Many patients with AIDS suffer a profound deficiency in cell mediated immunity. It is well known that development of competent T-cell immunity is thymus dependent. Therefore, four thymic tissues available from patients with AIDS were examined for possible M. fermentans cognitans infection. Two of the thymic tissues were described grossly at autopsy as inviolated thymus, one from a two year old and the other from a eight year old. Both of these pediatric patients contracted AIDS from blood transfusions.

The other two thymuses were derived from adult AIDS patients and the autopsy reports contained no specific gross tissue description. Immunohisto-chemical studies, using M. fermentans cognitans-specific monoclonal antibodies, showed positive immunoreaction in all four thymic tissues. Both mononuclear lymphohistiocytes and epitheloid cells were stained positively (FIG. 26).

FIG. 26 shows the immunohistochemistry of thymic tissues derived from patients with AIDS. FIG. 26A is a low-magnification photograph of a thymus immunostained by M. fermentans cognitans-specific monoclonal antibody (C42H10) (X71.5). FIG. 26B is a higher magnification of the positively immunostained lymphohistiocytes in the junction between cortex and medulla shown in 26A, left curve arrow (X715). FIG. 26C is a higher magnification of the positively immunostained lymphohistiocytes in the septal interstitial tissues in 26A, right curve arrow (X715). FIG. 26D is a low-magnification photograph of a thymus from another AIDS patient (X126.5). FIG. 26E is a higher magnification of the positively immunostained cells in 26D (X142).

Electron microscopic examination of the areas of the thymus with significant positive immunoreaction showed ultrastructurally many particles resembling mycoplasma. The particles were located both intracellularly in the cytoplasm of lymphohistiocytes (FIG. 27 A, B) and apparently free-growing extracellularly (FIG. 27 C, D). FIG. 27 shows an electron micrograph of an AIDS thymus immunostained positively for M. fermentans cognitans-specific antigens. FIG. 27A is an electron micrograph of mononuclear lymphohistiocytes with many intracytoplasmic electron dense mycoplasma-like particles (arrows) (N is the nucleus and bar represents 100 nm). FIG. 27B is a higher magnification of the electron dense mycoplasma-like particles in the cytoplasm of a mononuclear cell shown in 27A (P is a polysomal structure and bar represents 100 nm). FIG. 27C is an electron micrograph of many mycoplasma-like particles found both inside the membrane bound cytoplasmic vesicle (arrow heads) and also extracellularly in the interstitial tissue (arrows) (N is the nucleus with degenerating changes, Bar represents 100 nm).

FIG. 27D is a higher magnification of the extracellular mycoplasma-like particles. The outer limiting membrane of some particles (arrows) can be identified (Bar represents 100 nm).

Most of the nearly spherical particles measured 100–300 nm. No cell wall was associated with these particles. However, a prominent halo with a clear space surrounding each of these intracellular particles was commonly noted.

Occasional cells exhibited cytopathological changes and even appeared to be necrotic. However, most cells in these tissues were morphologically unremarkable. There was no tissues reactive process and/or an inflammatory reaction identified.

Liver

Ten livers from patients with AIDS who had unexplained abnormal liver function tests were examined. Work-ups for both hepatitis B and A infections were negative in these patients.

Four of these ten livers were positive by immunohistochemistry using M. fermentans cognitans-specific monoclonal antibodies. Histopathology of these four livers varied from no pathological changes except mild periportal infiltrates of lymphohistiocytes (two) to fulminant hepatocyte necrosis without any inflammatory reaction (one) and patchy areas of hepatocyte necrosis associated with prominent acute and subacute inflammation (one). The positively immunostained cells in these livers were the infiltrating inflammatory cells and the hepatocytes with or without any evidence of cytopathological changes (FIG. 28). Some areas of sinusoidal space lined by Kupffer cells were also stained positively.
FIG. 28 shows the immunohistochemistry of livers derived from patients with AIDS, using monoclonal antibody C42H10. FIG. 28A is a photomicrograph at a portal area in an AIDS liver with patchy areas of necrosis. Prominent infiltrates of chronic inflammatory cells and proliferation of bile ducts (arrows) are identified (X390). FIG. 28B is a higher magnification of the positively immunostained cells in 28A (X780). FIG. 28C is the same portal area shown in 28A in a subsequent tissue section immunostained by a nonspecific monoclonal antibody with the same isotype IgCl/k. Hemosiderin pigments (arrow heads) are noted (X390). FIG. 28D is an immunohistochemical photomicrograph of another AIDS liver. No necrosis or histopathological changes other than mild infiltrates of chronic inflammatory cells in the portal area (P) can be found in the liver (X390).

The areas of liver showing positive *M. fermentans* cognitius-specific antigens were also retrieved from the original paraffin blocks for ultra structural examination. Microorganisms with typical mycoplasma morphology were identified in all four livers. These mycoplasma-like microorganisms could be found intracellularly in the cytoplasm of mononuclear lymphohistiocytes, Kupffer cells and hepatocytes. Many of these microorganisms also lined up extracellularly along the walls of sinusoids (FIG. 29). For comparison, an electron micrograph of *M. fermentans* cognitius in the liver of a silvered leaf monkey, experimentally infected with this pathogen (Example 9) is shown in the insert of FIG. 29E.

FIG. 29 shows an electron micrograph of AIDS liver immunostained positively for *M. fermentans* cognitius-specific antigens. FIG. 29A is an electron micrograph of a periportal area of an AIDS liver with adjacent necrosis. N is the nucleus of a mononuclear lymphohistiocyte. R is red blood cells in the small vessel and the bar represents 500 nm. FIG. 29B is a higher magnification of the mycoplasma-like microorganisms found in the empty extracellular space and lining along the outer surface of the lymphohistiocyte shown in 29A. Many intracellular particles (arrow heads) can also be identified and are difficult to differentiate with the extracellular particles (P is the polysomal structure and the bar represents 1200 nm). FIG. 29C is a higher magnification of the mycoplasma-like microorganisms lining the outer surface of the lymphohistiocyte (Bar represents 100 nm). FIG. 29D is an electron micrograph of another AIDS liver which showed no evidence of histopathological changes except mild portal infiltrates of chronic inflammatory cells (N is the nucleus and the bar represents 400 nm). FIG. 33E is a higher magnification of the mycoplasma-like particles shown in 29D. The insert shows *M. fermentans* cognitius in 2% glutaldehyde fixed liver of experimentally infected silvered leaf monkey at the same magnification (Bar represents 100 nm).

Lymph node and spleen

Two lymph nodes surgically removed from AIDS patients showed reactive changes with follicular hyperplasia and foci of sinus histiocytosis. No areas of necrosis were identified. Positive immunohistochemical reactions were seen primarily within the endothelial cells lining the lymphatic sinus or the mononuclear lymphohistiocytes found in the sinus. Both nuclei and cytoplasm were stained positively. The typical staining patterns were similar to the results presented previously, using polyclonal rabbit antiserum (Lo, S-C et al., *Am. J. Trop. Med. Hyg.* 40, 213 (1989)).

Sections from four of six autopsy spleens without pathological changes stained positively with *M. fermentans* cognitius-specific monoclonal antibody. Mononuclear histiocytes and reticular cells in periaxial regions, mononuclear, reticular cells and lymphocytes in areas of red pulps were the positive cells which often revealed varying degrees of swelling or disruption. The strongly-stained nuclei and cytoplasm resembled inclusion bodies in the immunohistochemical reaction. Positively stained cells could also be identified in two additional splenic tissues with areas of prominent necrosis. The positive immunohistochemical reaction was concentrated at periphery of the necrosis (data not shown).

Characteristic ultrastructures with morphological features typical of mycoplasma were identified in all four spleens (including two with necrosis) and two lymph nodes which were retrieved for electron microscopy.

Brain

More than 60% of patients with AIDS are reported to have abnormal central nervous system (CNS) symptoms (Navaia, B. A. et al., *J. Neurol. 19, 517 (1986)*). Since most AIDS patients have serological evidence of HIV infection, the CNS diseases in these patients with AIDS have been called HIV encephalopathy.

Eight brains from patients with AIDS who had prominent clinical symptoms of CNS diseases without histopathological diagnosis of a specific infection in the brains at necropsy were examined. Two of these 8 brains had lesions of fulminant necrosis and karyorrhexis associated with both acute and subacute inflammations. Both of these brains were from intravenous drug abusers with AIDS. One of the other brains had subacute encephalitis with mononuclear cell infiltration but no necrosis. The remaining 5 brains showed only atrophy, gliosis and occasional microglial nodules without evidence of necrosis or inflammation.

All 3 brains with histopathological evidence of acute or subacute encephalitis stained positively for *M. fermentans* cognitius-specific antigens. FIG. 30 shows the positive immunostaining of the acute and subacute inflammatory cells in the periphery of a necrotic brain lesion.

FIG. 30A is a photomicrograph of the periphery of a necrotic cerebellar lesion immunostained positively by *M. fermentans* cognitius-specific monoclonal antibody (C42H10) (X390). FIG. 30B is a higher magnification of the periphery of the lesion in 30A and shows both acute and subacute inflammatory cells immunostained positively (X780). FIG. 30C is also a higher magnification of the positively stained cells in 30A (X780). FIG. 30D is a photomicrograph of the same periphery area of the necrotic lesion immunostained by a non-specific monoclonal antibody with the same isotype IgG1/k. Cells with prominent cytopathological changes and disruption (arrows) are evident (X780).

Furthermore, three of the 5 brains showing no evidence of inflammation or necrosis also revealed positive immunostaining. The positively stained cells showed degenerating changes, and often became inclusion body-like structures in the gray and white matter. The patterns and characteristics of positive immunohistochemical staining identified in these histologically unremarkable brains were comparable to those previously reported, using rabbit polyclonal antiserum (Lo, S-C et al., *Am. J. Trop. Med. Hyg.* 40, 213 (1989)).
Ultrastructural confirmation of *M. fermentans* incognitus infection in these 6 brains which immunostained positively for *M. fermentans* incognitus-specific antigens was also performed. Many electron-dense particles with features of mycoplasma organisms were identified extracellularly positively for *M. fermentans* incognitus-specific antigens was also performed. Many electron-dense particles with features of mycoplasma organisms were identified extracellularly or in the cytoplasm of mononuclear lymphohistiocytes located in the periphery of necrosis. Clusters of particles with morphological features of mycoplasma could also be identified in the encephalopathy AIDS brains showing positive immunostaining but with no evidence of necrosis and inflammation (FIG. 31). Some of the particles had prominent outer membranes. For comparison, the electron micrograph of *M. fermentans* incognitus with an apparent outer limiting membrane identified in cytoplasm of Sb51 cells in culture is shown in the insert of FIG. 31D.

FIG. 31A is an electron micrograph of mycoplasma-like particles (arrows) clustered together in the hippocampus. F is a bundle of neural filament and N is the nucleus of a mononuclear cell (Bar represents 100 nm). FIG. 31B is a higher magnification of the mycoplasma-like particles shown in 31A. The outer limiting membrane (small arrows) of some particles is prominent. (Bar represents 100 nm). FIG. 31C is a higher magnification of the same particles. FIG. 31D is a high magnification electron micrograph of mycoplasma-like particles found in the brain stem from another AIDS patient (large photo to right). The typical particles with well-preserved outer membrane (small arrows) are shown in an endothelial cell. Cytoplasmic membrane (large arrows) of the endothelial cells and basement membrane (arrow heads) of the vessel can be identified. L is the lumen of the vessel. The insert shows an electron micrograph of VLIA (*M. fermentans* incognitus) originally identified in the cytoplasm of Sb51 cells, at the same magnification. The unit membrane of *M. fermentans* incognitus (small arrows) is prominent in the well fixed (2% glutaldehyde) and well preserved culture specimens. Cytoplasmic membrane (large arrows) of the Sb51 cell is also identified (Bar represents 200 nm).

Placentas

Two placenta delivered at full term by two women with AIDS were available for study. The babies were reported to be normal at birth. However, no follow-up was available.

Histopathological examination showed occasional infiltrate of acute inflammatory cells in the chorionic plates in one of the placentas. The second placenta was histologically unremarkable. The special histopathological stains did not reveal any pathogens in either of the two placentas. Immunohistochemical study of both placentas, using *M. fermentans* incognitus-specific monoclonal antibodies C42H10 and D81E7, exhibited positive immunoreaction in areas of Hofbauer cells and stromal connective tissues in the chorionic vili (FIG. 32). Some decidual cells in the stratum basalis were also stained positively.

FIG. 32 shows the immunohistochemistry of a placenta delivered by a patient with AIDS. FIG. 32A is a photomicrograph of placenta tissue positively immunostained by a *M. fermentans* incognitus-specific monoclonal antibody (C42H10). The insert shows the same placental area in a subsequent tissue section immunostained by a non-specific monoclonal antibody with the same isotype IgG1/k (X 195). FIG. 32B is a higher magnification of the positively immunostained cells shown in 32A. The cytoplasm (arrow heads) or the surface of vacuolated cells (arrows) more often reveals positive reaction. Cells showing cytopathological changes with both nuclei and cytoplasm are positively stained (curve arrows) may resemble atypical inclusion bodies (X780).

Electron microscopic examination of the Hofbauer cells and connective tissues in the positively stained chorionic villi revealed numerous particles characteristic of mycoplasma (FIG. 33). Some particles identified in the Hofbauer cells were probably in membrane bound vesicles. Many microorganisms, with a wide variation of size, shape and electron density, appeared to focally colonize in the stomal connective tissue (FIG. 33). A prominent halo with a clear space surrounding each of these particles was often noted. No accompanying acute inflammatory cells or other reactive process was identified. Some apparently better preserved particles exhibited recognizable outer limiting membranes. However, many of the mycoplasma-like particles did not have definite outer unit membranes; they showed only an electron dense internal matrix with a fine granular configuration.

FIG. 33 shows electron microscopy of an AIDS patient's placenta immunostained a positively for *M. fermentans* incognitus specific antigens. FIG. 33A is an electron micrograph of a Hofbauer cell containing many mycoplasma-like particles in the cytoplasm. Some particles are apparently in the membrane bound cytoplasmic vesicles (arrows). N is the nucleus and I is a cytoplasmic inclusion body (Bar represents 800 nm). FIG. 33B is a higher magnification of the mycoplasma-like particles. Both spherical electron dense particles (arrow heads) and flask shape particles (arrows) typical for mycoplasma organisms are found to colonize in the stomal connective tissue (Bar represents 1000 nm). FIG. 33C is a higher magnification of the mycoplasma-like particles shown in 33C. Typical electron dense internal matrix with fine granular configuration of these particles is shown. Occasional particles contain recognizable outer membrane (arrows) (Bar represents 100 nm). FIG. 33D shows many of the particles are also those of less electron dense but with granular appearing internal matrix. These particles often have more prominent outer limiting membrane (arrows) (Bar represents 100 nm).

Detection of *M. fermentans* incognitus specific genetic material

*M. fermentans* incognitus DNA was identified in the tissues of thymus, liver and spleen from patients with AIDS as well as in the placentas delivered by two women with AIDS using the 35S labeled psb-2.2 probe. FIG. 34 shows positive labeling with grains heavily concentrated in cells of livers and spleen. Cytological and/or histological identification of the specific “types” of cells containing *M. fermentans* incognitus DNA, revealed that they were the Kupffer cells and hepatocytes in the liver showing minimal histopathological changes (FIG. 34A), the infiltrating lymphoid cells and histiocytes in portal tracts of another liver (FIG. 34C), and the lymphocytes in periarteriolar lymphoid sheaths (white pulp) of spleen (FIG. 34D).

In parallel, 35S-labeled M13 mp 19 vector DNA which did not contain *M. fermentans* incognitus DNA, did not elicit any positive signals in the consecutive
sections from these tissues (FIG. 34B). Five tissues of spleen and liver from three patients who died of non-AIDS conditions were used as negative controls and also did not reveal any evidence of positive signals.

FIG. 34 shows in situ hybridization for \textit{M. fermentans} incognitus nucleic acid in liver and spleen from patients with AIDS. FIG. 34A shows cells with strong labeling (arrows) are seen in an AIDS liver with no histopathological abnormality after hybridization with \textsuperscript{35}S labelled psb-2.2 DNA. Higher magnification (insert) reveals dense clusters of grains over individual hepatocytes or Kupffer cells (X240, X770). FIG. 34B is the same area of 34A in the consecutive tissue section, hybridized with \textsuperscript{35}S labelled cloning vector DNA not containing \textit{M. fermentans} incognitus DNA (X270). FIG. 34C shows lymphocytes and histiocytes with positive labeling seen in the portal tract infiltrated with mononuclear inflammatory cells in the liver of another AIDS patient (X770). FIG. 34D shows lymphocytes with strong labeling seen in the periarteriolar lymphoid sheath of the spleen. The central arteriole (Ar) is identified. The insert shows higher magnification of heavily concentrated grains over the lymphoid cells in this white pulp (X350, X770).

Kidney

Renal tissues from 203 patients who died of AIDS as defined by the Centers for Disease Control criteria were selected for study. The patients lived in various geographic locations including the continental United States (US), Puerto Rico (PR), Haiti, and Africa. The different racial backgrounds included in this study were white, black, Hispanic, and Oriental. Risk activities for AIDS were varied and included intravenous drug abuse (IVDA), homosexual contact, heterosexual contact, and history of blood transfusion. The patients had a wide range of opportunistic infectious agent including \textit{Pneumocystis carinii}, \textit{Toxoplasma gondii}, \textit{Candida albicans}, \textit{Cryptococcus neoformans}, \textit{Histoplasma capsulatum}, \textit{Mycobacterium avium-intracellulare}, \textit{M tuberculosis}, \textit{cytomegalovirus}, herpes simplex virus, and others.

Of the 203 total patients comprising this study, 20 patients had renal histopathologic changes characteristic of AIDS-associated nephropathy (AAN). Group B consisted of 15 patients selected from the remaining 183 who had no significant clinical or pathologic evidence of renal disease. These patients were matched as closely as possible with Group A patients in terms of the distribution of age, gender, race, and risk activities which Sections of kidney from the autopsies of 203 patients with AIDS, as well as renal tissues from the five (Group C) controls, were examined by conventional light microscopy. Special stains, including periodic acid-Schiff, Grocott's methenamine silver, Ziehl-Neelsen, mucicarmine, Masson's trichrome, and Brown and Hopps, were obtained to evaluate glomerular and tubular morphology as well as to document the presence of various opportunistic infections. For the 20 cases of AAN, glomerular, tubular, and interstitial changes were semi-quantitatively graded and recorded.

Renal tissues from 15 of the 20 patients from Group A and all of the tissues from Groups B and C were evaluated using monoclonal antibodies (MABs) against \textit{M. fermentans} incognitus as described above.

Formalin-fixed, paraffin-embedded sections of kidney were immunochemically stained with MABs against the incognitus strain, as previously described. Specific areas of positive staining were circled (approximately 1 mm in diameter) and removed from the matched paraffin tissue blocks. Tissues were then deparaffinized and processed as described above. After embedding all tissues in epoxy resin, semi-thin sections were cut and stained with alkaline toluidine blue for histologic analysis. The thin sections of the selected blocks were stained with lead citrate and uranyl acetate and examined by electron microscopy.

Light Microscopy

For all 20 cases of AAN, the earliest recognizable glomerular change consisted of relative and actual dilatation of Bowman's space, with concomitant capillary tuft wrinkling, compression, or complete collapse (FIG. 3a). Bowman's spaces often contained finely granular proteinaceous material which was also present in the lumens of adjacent proximal convoluted tubules. The subsequent glomerular change was "early" segmental or global glomerulosclerosis, as evidenced by hypertrophy and vacuolization of visceral epithelial cells and capillary endothelial cells, increased amounts of mesangial matrix material in either a segmental or global distribution, and small protein droplets within epithelial cells and Bowman's space. The most advanced glomerular change was fully evolved ("late") segmental and global sclerosis. In the latter case, glomerular tufts were transformed to round "sclerotic balls," sometimes surrounded by a rim of hypertrophic epithelial cells. In this advanced stage, homogeneous, dense cast material often filled the dilated Bowman's spaces and contiguous tubular lumens. The peripheral edges of these casts had scalloped borders, created by side by side "holes" in the cast material adjacent to tubular epithelial cells.

Tubular changes usually paralleled glomerular changes. In early stages, tubular epithelial cells with cytoplasmic swelling contained many protein droplets. Subsequently, tubular lumens became dilated and contained protein droplets or granular proteinaceous material, as well as degenerated sloughed epithelial cells. In later stages, tubules showed microcystic dilatation and were filled by dense cast material. Epithelial cells within such tubules were flattened by compression by the large proteinaceous casts. In all cases, variable degrees of interstitial edema and mononuclear cell inflammation were present. Special tissue stains did not reveal any evidence of infection with bacteria, fungi, or mycobacteria in these kidneys.

Sections of renal tissue from the 15 group B patients showed minimal structural abnormalities including focal mild mononuclear cell infiltration of the interstitium, rare mononuclear cells within glomerular capillary lumens, and occasional hyaline casts. Renal tissue from three of the five group C patients also demonstrated normal histology, renal tissue from the remaining two showed changes consistent with reflux nephropathy and moderate to marked nephrosclerosis, respectively.

Immunohistochemistry

For all of the 15 group A patients, there was positive staining by M incognitus-specific MABs in several locations including glomerular endothelial and epithelial cells, capillary basement membrane, tubular epithelial cells, tubular casts, and mononuclear interstitial cells. Although all cases had positive staining for antigens of this microorganism in the above locations, six cases showed more prominent positivity in glomerular epithelial and endothelial cells, while nine cases had
greater positivity in tubular epithelium and casts. Particularly intense staining could often be seen in partially degenerated cells within the casts, or within the amorphous cast material itself.

Kidney tissues from group B patients showed positive staining for incognitus strain-specific antigens only within occasional mononuclear interstitial cells. These mononuclear cells were either histiocytes or lymphocytes. None of the cases in this group demonstrated positivity within the glomerulus or tubules. The renal tissues of group C patients showed no staining for incognitus strain-specific mycoplasmal antigens in any locations.

**Electronmicroscopy**

Electron microscopic examination of tissues from the particular areas highly positive for incognitus strain-specific antigens revealed structures strongly resembling mycoplasmal organisms in various locations in all 15 group A cases.

In seven patients, mycoplasma-like structures (MLS) were identified in glomerular endothelial cytoplasm and in the adjacent capillary basement membrane. Such endothelial cells often displayed enlargement and vacuolization, with MLS sometimes localized in clusters within the vacuoles.

Although 12 patients showed MLS within the glomerular basement membrane, seven patients, with more intense immunoperoxidase staining for the mycoplasmal antigens within this location demonstrated greater involvement of the membrane on electron microscopy. Mycoplasma-like structures could be seen in sub endothelial, intramembranous, and subepithelial locations with accompanying membranopathies. These changes consisted of (1) small holes in the basement membrane surrounding intramembranous MLS, (2) splits and large irregular defects in the membrane associated with scattered MLS, (3) thickening of the membrane, associated with intramembranous MLS, and (4) complete breaks in the basement membrane in areas of heavy MLS infiltration.

Mycoplasma-like structures were also present within visceral epithelial cells which often displayed cytoplasmic degeneration, vacuolization, and partial detachment from the underlying basement membrane. In many instances these cells were completely detached from the basement membrane, embedded in proteinaceous cast material within Bowman's space.

Numerous MLS were likewise found within the contiguous large proteinaceous casts in microscopically dilated tubules. Morphologically, these particles varied from spherical electron-dense forms to large ovoid, flask-shaped or undulating forms. Mycoplasmal-like structures were present in great numbers in detached, degenerated tubular epithelial cells, which were often incorporated into the casts.

Electron microscopic study of renal tissues of 10 of the 15 group B cases showed occasional mononuclear interstitial cells containing MLS. Group C cases displayed no MLS ultrastructurally. Glomerular endothelial tubuloreticular inclusions were present in the 15 group A and 10 group B cases, and were absent in the five group C cases.

In this study, we have identified mycoplasmal infection of the parenchymal cells in kidneys of AIDS patients with typical histologic changes of AAN. There is good correlation between the immunohistochemical presence of the incognitus strain mycoplasmal antigens in visceral epithelial and tubular epithelial cells demonstrating the cytopathic changes typical of AAN, and the ultrastructural presence of MLS within the same critical cells. The same correlation also holds true for other microscopic locations, such as glomerular endothelial cells and renal tubular casts. The ultrastructural finding of significant numbers of MLS within the glomerular capillary basement membrane with evidence of membranopathogenic effect can be of particular importance when considering the pathogenesis of this nephropathy.

In summary, this study documents a spectrum of renal histopathologic changes which helps further delineate the morphogenesis of AAN. The study has also demonstrated the mycoplasmal infection of glomerular endothelium, epithelium, and basement membrane, as well as tubular epithelium, in the kidneys of AIDS patients with AAN. Infection of these functional parenchymal cells by *M. fermentans* (incognitus strain) may have contributed to the development of glomerulosclerosis, proteinuria, intratubular casts, and renal failure in these patients with AIDS.

**EXAMPLE 24**

Enhancement of HIV-1 Cytocidal Effects in CD4+ by *M. fermentans* incognitus

The effects of the *M. fermentans* incognitus on HIV-1 infection of a CD4+ human T lymphocyte cell line, designated previously as A3.01 (Folks, T. et al., Science 231, 601 (1986)).

Normally, HIV-1 infection of human T lymphocytes in vitro produces pronounced cytopathic effects (CPE) with the release of newly replicated virus (Lifson et al., Science 232, 1123 (1986)). The formation of large multinucleated cells, termed syncytia, and high levels of reverse transcriptase (RT) activity is a characteristic feature of HIV-1 infection in vitro (Lifson et al., Nature 323, 725 (1986)). A3.01 cells (5×10⁷) were infected with (A) HIV-1 (1×10⁶ infectious units) and incognitus strain (1×10⁵ infectious units), (B) HIV-1, or (C) incognitus strain. Cells in each culture were incubated at 37°C for 2 hours and then washed once with RPMI 1640 medium. The infective titer of HIV-1 was previously determined by exposing A3.01 cells to tenfold serial dilutions of HIV-1 culture stock for 2 hours at 37°C. The highest dilution in which the presence of RT activity could be detected after 14 days in culture represented one infectious unit. We grew the incognitus strain in modified SP-4 media and filter-clone it three times from a single colony on agar plates. The organisms were washed once and resuspended in RPMI 1640. The titer of incognitus strain after infection of NIH 3T3 cells was determined by antigen dot blot assay. The cell cultures were maintained with RPMI 1640 supplemented with 10% FBS. Large numbers of syncytia formed when HIV-1 alone infected A3.01 cells, but syncytium formation disappeared in A3.01 cells simultaneously infected with HIV-1 and incognitus strain (Fig. 35) despite clear evidence of a cytocidal effect. Results are the average of the number of syncytia per field (X200) of ten fields examined per culture. The error bars indicate standard deviation of the mean.

The cytocidal effect and inhibition of RT activity in HIV-1 infected A3.01 cells by *M. fermentans* incognitus was analyzed. A3.01 cells were cultured after (B) infection by HIV-1, (A) infection by HIV-1 and incognitus strain, (D) infection by incognitus strain, or (E) no treatment. Each point on each graph is the
average of the results of three independent cultures. (A) Cell viability was determined with the Trypan blue exclusion test with a total of 200 cells counted for each time point. (B) Samples of culture supernatants were tested daily with the standard RT enzyme assay using the incorporation of tritiated triphosphate nucleotides (Baltimore et al., Proc. Natl. Acad. Sci. USA 68, 1507 (1971). Conditions of HIV-1 and mycoplasma infectious were the same as described above. The culture infected by mycoplasma alone [indicated by Δ in (A)] also had no detectable RT activity. The M. fermentans incognitus significantly enhanced the cytotoxic effects of HIV-1 infection in A3.01 cells (FIG. 36A). Furthermore, populations of cells that had been infected by HIV-1 alone gradually recovered from the initial cytotoxic effect and remained persistently infected. In contrast, A3.01 cells infected by both HIV-1 and incognitus strain died. In this study, incognitus strain infection alone did not produce detectable cytotoxicity. As expected, culture supernatants from A3.01 cells infected with HIV-1 had clear RT activity. However, samples from the coinfected cell culture showed little or no RT activity (FIG. 36B).

Despite the absence of RT activity, virus-specific protein synthesis and assembly was occurring. This activity was shown by examining culture supernatants. Culture supernatant (100 ul) was tested for the presence of viral antigen (HIV-1 antigen assay kit, Integrated Diagnostics, Gaithersburg, Md.). The assay kit uses an enzyme-linked immunosorbent assay (ELISA) technique, and the procedures performed in this study were in strict accordance to the instructions supplied with the kit. The negative control (phosphate-buffered saline) had an absorbance (A406) reading of less than 0.1 at 410 nm. Each point on the graph (FIG. 37A) is the average of the results of three independent cultures. (●) A3.01 + HIV-1, (▲) A3.01 + HIV-1 + incognitus strain, (●) A3.01 (FIG. 37B) shows an electron micrograph of a cell culture infected simultaneously with both HIV-1 and incognitus strain. Numerous viral particles are seen in this culture with lytic cells. Occasional electron-dense forms of incognitus strain (arrows) can also be seen. Bar = 400 nm. The coinfected cell culture produced HIV-1-specific p24-p25 rapidly as the culture infected by HIV-1 alone (FIG. 37A). Electron microscopy of coinfected cells showed typical HIV virions (FIG. 37B). The assembled virions were infectious. Supernatant from the coinfected culture, which showed no detectable RT activity, was tenfold serially diluted and incubated with fresh A3.01 cells. We found comparable infectious units of HIV-1 (10⁵ per milliliter) to be produced in the supernatants after infection of cell cultures either by HIV-1 alone or by both HIV-1 and incognitus strain (See, Lo et al., Science 251, 1074 (1991)).

To test if substances in cultures infected by incognitus strain directly affected the RT enzyme assay, culture supernatant from A3.01 cells coinfected with HIV-1 and incognitus strain was mixed with the culture supernatant containing HIV with known RT activity. Over 90% of the RT activity was inhibited when less than a third of the active supernatant was replaced by culture supernatants containing both HIV-1 and incognitus strain. Enzyme inhibition occurred immediately, and prior incubation of the mixture of culture supernatants was not required. We observed a comparable degree of inhibition when we used culture supernatant from A3.01 cells infected with only incognitus strain in the inhibition assay. Thus, the results can be best explained by the presence of some mycoplasma product or products in the assay lysate which directly interfered with the RT assay. Some mycoplasmas have recently been found to produce highly active nucleases (Marcus et al., J. Cell Physiol 143, 416 (1990), which could potentially be involved.

The masking effect of HIV RT activity may not be unique to incognitus strain. Suppression of HIV RT has recently been reported in M. hyorhinis-contaminated lymphocyte cultures (Vasdevachari et al., AIDS Res. Hum. Retroviruses 6, 411 (1990). But in contrast to the results in this report, the HIV-1-infected cultures contaminated by the swine mycoplasma still formed prominent syncytial cells. Our study indicates that syncytium formation and the actual cytotoxic effect can be separate events. Our findings support the earlier reports (Schocki, et al., Nature 322, 470 (1986); Somayajulu, et al., J. Virol 61, 3114 (1987) that state that the formation of syncytial cells is not a necessary prerequisite for proliferation of HIV-1.

It has recently been shown that nontoxic doses of the antibiotic tetracycline may significantly reduce the cytotoxic effects of HIV-1 (Lemaitre, et al., Res. Virol. 141, 4 (1990). The tetracycline-treated cultures continued to produce a high titer of HIV-1. The authors suggested that a catabolic agent, most likely a mycoplasma, was involved with the cytotoxic effect observed in the HIV-infected cultures. Indeed, additional study and characterization from their laboratory has confirmed that the hidden agent in the cultures is a mycoplasma (Wright, Science 248, 682 (1990)).

Researchers from Japan have reported that just the antigens of killed mycoplasma (Acholeplasma laidlawii) could stimulate HIV-1 production (p24 antigen and infectious particles) in HIV-1-infected cells (Chorodhur et al., Lancet 336, 247 (1990). In our study, approximately equivalent amounts of HIV antigen or infectious particles were produced in HIV-infected or HIV and incognitus strain-infected cultures despite significant differences in the numbers of viable cells. Thus, more HIV-1 may actually have been produced per individual cell in the coinfected culture; this finding is similar to the findings of the Japanese researchers.

AIDS patients can be infected with a number of pathogenic microbes and frequently are systemically infected with the incognitus strain (Lo et al., Am. J. Trop. Med. Hyg. 40, 213 (1989); Lo et al., Jid 41, 601 (1989). Thus, the observation that coinfection by incognitus strain profoundly enhances cytotoxic effects of HIV-1 infection in vitro.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. The description of the invention is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention, and includi such departures from the present disclosure as come within known and customary practice within the art to which the invention pertains.
(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 17

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 22 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(i) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
   (A) ORGANISM: Mycoplasma fermentans
   (B) STRAIN: incognitus

(v) IMMEDIATE SOURCE:
   (B) CLONE: RS 48 Probe

(x) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 220 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(i) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
   (A) ORGANISM: Mycoplasma fermentans
   (B) STRAIN: incognitus

(v) IMMEDIATE SOURCE:
   (B) CLONE: phb 2.2

(x) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GATAAGATGG AAAAAAAG AAAGGATTAT TTTGACCTTT TTAACTATAA TAAAAGACTA 780
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69

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| AAAAGTGGG AACAGATGGT AGCTGATTTA AATTTAAAAG TGTGAAAAAG GCATATTTTT 1080 |
| TCTCTGTAGT TGATTTTGGAA AACAGAGAGA TTTTAGGTAA TTTGAGTTTTT AAAATGCTA 1140 |
| ATTTAAGAAT GAGTGGTTAA ATGTTAGAAA AGCTGAGAGA GAAATGCCCAC AGCTTTAAA 1200 |
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| TAGAAGAAAA ACAAAACAAT CAAAGCATGT CAAGAAGAGG AAATTGTTTA GACAAATATG 1320 |
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| TTAATAGTGT TAAAGAATTTT AAAACTGTCT TTAGAGATAT ATTTCATATT AAATAATAG 1440 |
| CAGAATGTGT AATAAAATGA AAGACCTTAG TCTGTTATTAC ACAGGAATAA GCTCAACAT 1500 |
| AATGAAAAAG TCCAAATTTC GGGGTCATA CAATTTTTG TGAATTTTCT TTTTGCCCAA 1560 |
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1405 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(v) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:

(A) ORGANISM: Mycoplasma fermentans
(B) STRAIN: incognitus

(vi) IMMEDIATE SOURCE:

(B) CLONE: IS element

(x) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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| AAAATTATCG GAAAGCAATG ATATTTTTCG GCAGAAAGCT GAGAAAGAAA ATATAATATT | 780 |
| CTTTATCAA AGGTGATGCT CACAAACAT TCCAAAACCTG CTTTTAGATA AAGAAATATC | 840 |
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| GGAACAGATG TTACTGAAAT TAAATTAAAA AATGATGAAA AGCATATTCT TTCTCTGTAT | 960 |
| GTGGATTTCG AAAAAAGAGA GATTTTGAGT TATTCGATT CTAAAATGCC TAATTTAAGA | 1020 |
| ATGGTTGCTA AAAATGTTAGA AAACGTAGAA GAGAATGGCC ACAGCTAAAA AAATGATTA | 1080 |
| TTACATACCTG ATCAAAGGAT AGCAATACCT CATCAAAGGAT ATATTGATTA TTTGAAGAA | 1140 |
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| TTTAAAGAAT TTTAAACTCG TTTAGGAGAT ATATTTTCAT TTATATAATG GACAGAATGG | 1320 |
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 29 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
   (A) ORGANISM: Mycoplasma fermentans
   (B) STRAIN: incognitus

(vi) IMMEDIATE SOURCE:
   (B) CLONE: left inverted repeat

(x) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 29 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
   (A) ORGANISM: Mycoplasma fermentans
   (B) STRAIN: incognitus

(vi) IMMEDIATE SOURCE:
(B) CLONE: right inverted repeat

(xii) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AAAAAGTCCA AAAAAAGGGG TCCATACCA 29

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 429 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
   (A) ORGANISM: Mycoplasma fermentans
   (B) STRAIN: incognitos

(vi) IMMEDIATE SOURCE:
   (B) CLONE: ORF-1

(xiii) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATGCAATTTA AATTAAAAA AGTAAAGA AAAAAATGAA ATAGAGATAT AAAAGGTTAT 60
TTAAATTAA AACTTGATCA AAAAGATAAA ATTATCGAT TATATTTCG AGAATTTAT 120
ATTTTAGAAA TATCTAAAAAT AATGGAAGC TCTTATTCAG CATGCTATTC AGTAAATGAA 180
AAATTAAAGG AATTGTTGTA TAATTTTGGT GTATGGAAG AGAAAAAAGG AAGAAAAATCT 240
AAATTAATTG TAGATTCTGA AAAAGGCAAA AATTAAAAA TCAATATTGA AATAAAAATA 300
GAAAATAGG ATTTAATTT TAAACATATT AACGAAGAGG ATAAAATCT CAAATGGGAG 360
AATGCAGATT GAAAAAGGT GAGCGCTTGT TTTCAATTTA AAGACTCAGT ACACAAAAGAA 420
AATTCAAAA 429

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 309 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
   (A) ORGANISM: Mycoplasma fermentans
   (B) STRAIN: incognitos

(vi) IMMEDIATE SOURCE:
   (B) CLONE: ORF-2

(xiv) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATGTTTGGTA AAAATTTTGA AAAAGTAGAA GAGAATGGCC ACACGCTAAAA AAATGTATTA 60
TTCAATCTCG ATCAAGGATG ACAATACACT CATCAAGATT ATATGATTAT TTTGAAGAA 120
AAACAACCAA CTCAGAGCAT GTCAAGAAAG GAAATGTGTT TAGACAATAG TCTACTGAA 180
TGTTTATTTA GTGTATTTAA AAGAGAATTT TGATTTGGAAG AGAGAAAAGA ATTTAATGAT 240
TTAAAGAATT TTTAATCTGC TTTAGGAGAT ATATTTCTCA TTATAAATAT GACAAGATTTG 300
TTATAAAT 309
(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) ORIGINAL SOURCE:
(A) ORGANISM: Mycoplasma fermentans
(B) STRAIN: incognitus

(vi) IMMEDIATE SOURCE:
(B) CLONE: ORF-3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

ATGAATATTA AAGCTTTTGG ACTCTATTTT ATTTTTATGA TAATTCATT GATCATAACT 60
GTAATCGTCT ATGCTGGACA AAATAAAGCG GCACCATCAA TCACATAC AACTTTACTT 120
TGAGTTTTAA TTTGTTCTTT TCAATATCT ACAATCTCTT CTCATTATTT ATTAATTITA 180
TTTTTCATTTG AATATGGTTT AATCAAAAA ATAGGCTCTT AAAATCAGA ACAAGAATA 240
GAAGCATCTA TAAGAAAATT TTGTTAATTT GCGATT 276

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 143 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycoplasma fermentans
(B) STRAIN: incognitus

(vii) IMMEDIATE SOURCE:
(B) CLONE: ORF-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met Gln Phe Lys Phe Lys Lys Val Lys Arg Asn Lys Trp Asn Arg Asp 1 5 10 15
Ile Lys Gly Tyr Leu Lys Leu Lys Leu Asn Gln Lys Ile Lys Ile Ile 20 25 30
Glu Leu Tyr Phe Gln Glu Phe Ser Ile Leu Glu Ile Ser Lys Ile Met 35 40 45
Glu Asn Ser Tyr Ser Ala Cys Tyr Ser Val Ile Glu Lys Tyr Lys Lys 50 55 60
Phe Gly Tyr Asn Ser Phe Ala Met Glu Lys Lys Gly Arg Lys Ser 65 70 75 80
Lys Ile Asn Leu Asp Ala Gln Lys Ala Thr Asn Phe Lys Ile Asn Ile 85 90 95
Glu Asn Lys Ile Glu Asn Lys Asp Leu Leu Ile Lys Gin Leu Lys Glu 100 105 110
Glu Asn Lys Ile Leu Lys Leu Glu Asn Ala Ile Ala Lys Lys Val Ser 115 120 125
Ala Leu Val Gln Leu Lys Asp Ser Leu Thr Lys Lys Asn Ser Lys 130 135 140

(2) INFORMATION FOR SEQ ID NO: 30:
-continued

### 1) SEQUENCE CHARACTERISTICS:
( A ) LENGTH: 103 amino acids  
( B ) TYPE: amino acid  
( D ) TOPOLOGY: linear

#### (ii) MOLECULE TYPE: peptide

#### (vi) ORIGINAL SOURCE:
( A ) ORGANISM: Mycoplasma fermentans  
( B ) STRAIN: incognitus

#### (vii) IMMEDIATE SOURCE:
( B ) CLONE: ORF-2

#### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Val Gly Lys Met Leu Glu Asn Val Glu Glu Asn Gly His Ser Leu</td>
<td>1</td>
</tr>
<tr>
<td>Lys Asn Val Leu Leu His Ser Asp Gin Gly Trp Gin Tyr Thr His Gin</td>
<td>20</td>
</tr>
<tr>
<td>Asp Tyr Ile Lys Tyr Leu Lys Glu Lys Gin Thr Thr Gin Ser Met Ser</td>
<td>35</td>
</tr>
<tr>
<td>Arg Lys Gly Asn Cys Leu Asp Asn Ser Pro Thr Glu Cys Leu Phe Ser</td>
<td>50</td>
</tr>
<tr>
<td>Val Ile Lys Arg Glu Phe Trp Phe Gly Glu Glu Lys Lys Phe Asn Ser</td>
<td>65</td>
</tr>
<tr>
<td>Phe Lys Glu Phe Lys Thr Ala Leu Gly Asp Ile Phe His Ile Ile Ile</td>
<td>85</td>
</tr>
<tr>
<td>Met Thr Glu Leu Leu Ile Asn</td>
<td>100</td>
</tr>
</tbody>
</table>

### 2) INFORMATION FOR SEQ ID NO:11:

#### (i) SEQUENCE CHARACTERISTICS:
( A ) LENGTH: 92 amino acids  
( B ) TYPE: amino acid  
( D ) TOPOLOGY: linear

#### (iii) MOLECULE TYPE: peptide

#### (v) ORIGINAL SOURCE:
( A ) ORGANISM: Mycoplasma fermentans  
( B ) STRAIN: incognitus

#### (vi) IMMEDIATE SOURCE:
( B ) CLONE: ORF-3

#### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Asn Ile Lys Ala Phe Gly Leu Leu Phe Ile Phe Met Ile Ile Leu</td>
<td>1</td>
</tr>
<tr>
<td>Leu Ile Ile Thr Val Ile Ala Tyr Ala Trp Gin Asn Lys Ala Ala Pro</td>
<td>20</td>
</tr>
<tr>
<td>Ser Ile Thr Tyr Thr Thr Leu Leu Trp Val Leu Ile Cys Val Phe Ser</td>
<td>35</td>
</tr>
<tr>
<td>Ile Leu Thr Ile Leu Ser Leu Tyr Leu Leu Ile Leu Phe Phe Ile Gly</td>
<td>50</td>
</tr>
<tr>
<td>Tyr Gly Leu Ile Lys Lys Ile Gly Leu Lys Lys Ser Gly Gin Gly Ile</td>
<td>65</td>
</tr>
<tr>
<td>Gly Ala Ser Ile Arg Lys Phe Val Lys Phe Ala Ile</td>
<td>85</td>
</tr>
</tbody>
</table>

### 2) INFORMATION FOR SEQ ID NO:12:

#### (i) SEQUENCE CHARACTERISTICS:
( A ) LENGTH: 57 amino acids  
( B ) TYPE: amino acid  
( D ) TOPOLOGY: linear
(iii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Escherichia coli

(vij) IMMEDIATE SOURCE:
(B) CLONE: IS3

(x.i) SEQUENCE DESCRIPTION SEQ ID NO:12:

Asn Val Ile Val His Thr Asp Arg Gly Gly Gln Tyr Cys Ser Ala Asp  
1 5 10 15
Tyr Gin Ala Gin Leu Lys Arg His Asn Leu Arg Gly Ser Met Ser Ala  
20 25 30
Lys Gin Cys Cys Tyr Asp Asn Ala Cys Val Glu Ser Phe Phe His Ser  
35 40 45
Leu Lys Val Glu Cys Ile His Gly Glu  
50 55

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycoplasma fermentans
(B) STRAIN: incognitus

(vii) IMMEDIATE SOURCE:
(B) CLONE: RS 47 Primer

(x.i) SEQUENCE DESCRIPTION SEQ ID NO:13:

GAATTCCTTTA ATGAGTTCG TC  22

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mycoplasma fermentans
(B) STRAIN: incognitus

(vii) IMMEDIATE SOURCE:
(B) CLONE: RS 49 Primer

(x.i) SEQUENCE DESCRIPTION SEQ ID NO:14:

TCCAAAAGT CCGAATTTG GGG  23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
( B ) TYPE: nucleic acid
( C ) STRANDEDNESS: single
( D ) TOPOLOGY: linear

(i i ) MOLECULE TYPE: DNA (genomic)

(i i i ) HYPOTHETICAL: NO

(i v ) ANTI-SENSE: NO

(v i ) ORIGINAL SOURCE:
- ( A ) ORGANISM: Mycoplasma fermentans
- ( B ) STRAIN: incognitus

(v i i ) IMMEDIATE SOURCE:
- ( B ) CLONE: RW004 Primer

(x i ) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACTATTGT CTAAAACATT TCCC

(2 ) INFORMATION FOR SEQ ID NO:16:

(i ) SEQUENCE CHARACTERISTICS:
- ( A ) LENGTH: 24 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

(i i ) MOLECULE TYPE: DNA (genomic)

(i i i ) HYPOTHETICAL: NO

(i v ) ANTI-SENSE: NO

(v i ) ORIGINAL SOURCE:
- ( A ) ORGANISM: Mycoplasma fermentans
- ( B ) STRAIN: incognitus

(v i i ) IMMEDIATE SOURCE:
- ( B ) CLONE: RW005 Primer

(x i ) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTTATTGCA TTTCTAAATC GCCT

(2 ) INFORMATION FOR SEQ ID NO:17:

(i ) SEQUENCE CHARACTERISTICS:
- ( A ) LENGTH: 24 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

(i i ) MOLECULE TYPE: DNA (genomic)

(i i i ) HYPOTHETICAL: NO

(i v ) ANTI-SENSE: NO

(v i ) ORIGINAL SOURCE:
- ( A ) ORGANISM: Mycoplasma fermentans
- ( B ) STRAIN: incognitus

(v i i ) IMMEDIATE SOURCE:
- ( B ) CLONE: RW006 Probe

(x i ) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCTGTTGCCA TTCTTCTTA CGTT

What is claimed is:
1. A biologically pure mycoplasma isolated from tissues of patients with AIDS comprising the mycoplasma produced by the cell line ATCC No. CRL 9127.
2. A biologically pure mycoplasma having the identifying characteristics of M. fermentans incognitus, ATCC 53949.
FIG. 17A

CPM

g/mL

FRACTION NUMBER
FIG. 17B

CONTROL
1-12
13-24
VLIA INFECTED
1-12
13-24

FIG. 18A

CONTROL
1-12
13-24
VLIA INFECTED
1-12
13-24

FIG. 18B

CONTROL
1-12
13-24
VLIA INFECTED
1-12
13-24
FIG. 35
FIG. 37A

Elisa (OD_{410})

Day of Culture