US Army Mycoplasma Fermentans Incognitus Patent

---------- United States Patent 5,242,820 Lo September 7, 1993 ---------- Pathogenic mycoplasma 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. ---------- Inventors: Lo; Shyh-Ching (Potomac, MD) Assignee: American Registry of Pathology (Washington, DC) Appl. No.: 710361 Filed: June 6, 1991

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Primary Examiner: Nucker; Christine M. Assistant Examiner: Preston; D. R. Attorney, Agent or Firm: Venable, Baetjer, Howard & Civiletti ---------- Goverment Interests
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The invention described herein was made in the course of work under a grant or award from the United States Department of the Army. ---------- Parent Case Text
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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.
---------- Claims

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.
---------- Description

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 nycoplasma is referred to hereinafter as the incognitus strain or M. fermentans incognitus.

The invention also relates to 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 .mu.m or even 0.22 .mu.m.

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.degree.-37.degree. C. Many species of Mycoplasma produce weak or clear haemolysis which appears to be due to the secretion of H.sub.2 O.sub.2. This H.sub.2 O.sub.2 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 a homologous M. fermentans 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 coccoid 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, 332 (1889) and Krass, C. J. et al., Int. J. Syst. Bacteriol. 23, 62 (1973)). Frank, after careful microscopic observation, began writing about invasion of plants (legume) by these microorganisms and stated: "the changed 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. Jahrb. 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 (>10.sup.9 /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 protoplasm 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 at., Ann.Neuro 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)).

Through co-cultivation of AIDS patients' peripheral blood cells with mitogen-stimulated normal human lymphocytes or permanent human T-cell lines, a number of laboratories have isolated T-cell-tropic human retroviruses (HTLV-III/LAV), Barre-Sinoussi, F., et al., Science 220, 868 (1983); Gallo, R. C., et al., Science 224, 500 (1984). Epidemiologically, the newly isolated retroviruses have been shown to be highly associated with patients of AIDS and/or AIDS-related complex (ARC). Schupback, J., et al., Science 224, 503 (1984); Sarngadharan, M. G., et al., Science 224, 506 (1984). In vitro studies with HTLV-III/LAV have demonstrated T-cell tropism and cytopathic changes. Barre-Sinoussi, F., et al., supra; Popovic, M., et al., Science 224, 497 (1984). HTLV-III/LAV is believed to be the causative agent of AIDS.

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 unusual 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 ongonege 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 mycoplasama 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 fulminant 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 mycoplasma 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 innoculation 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 blot analysis of sucrose gradient-banded M. fermentans incognitus in which the blot was probed with .sup.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 pEH-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 pT 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 antiserum. 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 antiserum. 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 antiserum. 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.sub.1 shows an electron micrograph of the margin of necrosis of an adrenal gland highly positive for M. fermentans incognitus-specific antigens. FIG. 24A.sub.2 is a higher magnification of FIG. 24A.sub.1. FIG. 24B.sub.1 shows an electron photomicrograph of the peripheral zone of necrosis in lymph node highly positive for M. fermentans incognitus-specific antigens. FIG. 24B.sub.2 shows an electron photomicrograph of the peripheral zone of necrosis in lymph node highly positive for M. fermentans incognitus-specific antigens. FIG. 24B.sub.3 is higher magnification of FIG. 24B.sub.1. FIG. 25A shows analysis and comparison of DNA restriction patterns of VLIA and M. fermentans incognitus probed with psb-8.6. FIG. 25B shows analysis and comparison of DNA restriction patterns of VLIA and M. fermentans incognitus probed with psb-2.2. FIG. 26A shows the immunohistochemistry of thymic tissues derived from patients with AIDS. FIG. 26B is a higher magnification of FIG. 26A. FIG. 26C is a higher magnification of FIG. 26B. FIG. 26D shows the immunohistochemistry of thymic tissues derived from patients with AIDS. FIG. 26E is a higher magnification of FIG. 26D. FIG. 27A 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 mononuclear lymphohistiocytes. FIG. 27C shows an electron micrograph of an AIDS thymus immunostained positively for M. fermentans incognitus-specific antigens showing mycoplasma-like particles. FIG. 27D shows an electron micrograph of an AIDS thymus immunostained positively for M. fermentans incognitus-specific antigens showing mycoplasma-like particles. FIG. 28A 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 monoclonal antibody C42H10. FIG. 28C shows the immunohistochemistry of livers from patients with AIDS using a non-specific monoclonal antibody. FIG. 28D 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 is a higher magnification of FIG. 29A. FIG. 29C is a higher magnification of FIG. 29B. FIG. 29D shows an electron micrograph of AIDS liver immunostained positively for M. fermentans incognitus-specific antigens at low magnification. FIG. 29E is a higher magnification of FIG. 29D. FIG. 30A shows the immunohistochemistry of a brain derived from a patient with AIDS using monoclonal antibody C42H10. 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 incognitus-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 incognitus specific antigens showing Hofbauer cell. FIG. 33B shows electron microscopy of an AIDS patient's placenta immunostained positively for M. fermentans incognitus specific antigens showing Hofbauer cell. FIG. 33C shows electron microscopy of an AIDS patient's placenta immunostained positively for M. fermentans incognitus specific antigens showing stronal connective tissue. FIG. 33D shows electron microscopy of an AIDS patient's placenta immunostained positively for M. fermentans incognitus specific antigens showing stronal connective tissue. FIG. 33E shows electron microscopy of an AIDS patient's placenta immunostained positively for M. fermentans incognitus specific

antigens showing stronal connective tissue. FIG. 34A shows in situ hybridization for M. fermentans incognitus nucleic acid in liver from patients with AIDS. FIG. 34B shows in situ hybridization for M. fermentans incognitus nucleic acid in liver from patients with AIDS. FIG. 34C shows in situ hybridization for M. fermentans incognitus nucleic acid in spleen from patients with AIDS. FIG. 34D shows in situ hybridization for M. fermentans incognitus nucleic acid in spleen from patients with AIDS. FIG. 35 shows the inhibition of HIV-1-induced syncytium formation by M. fermentans incognitus. FIG. 36A shows the augmentation of cytocidal effect and inhibition of RT activity in HIV-1 infected A3.01 cells cultures by M. fermentans incognitus. FIG. 36B shows the inhibition of RT activity in HIV-1 infected A3.01 cell cultures by M. fermentans incognitus. FIG. 37A shows continued viral production of HIV-1 and M. fermentans incognitus in culture supernatant by ELISA. FIG. 37B shows continued viral production of HIV-1 and M. fermentans incognitus 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 nepthropathy, heart failure of AIDS cardiomyopathy, respiratory distress syndrome and infections and uncommon malgnancies 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 minimus.

B. Previous Related Applications

The present invention relates to a novel strain of infectious mycoplasma (M. fermentans incognitus) 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 (VLIA), 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 VLIA 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 incognitus) 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 incognitus, 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 cytolytic 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 ssay. 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.

However, the incognitus strain differs from M. fermentans in that it appears to be is more fastidious in its cultivation requirements 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 pleophorphic microorganisms with trilaminar outer unit membrane as designated by the arrows. The bars 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 .mu.m and 20 .mu.m, 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 .mu.g) 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 (PG18 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 raisedspecifically 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. fermentas 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 .mu.g) 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. pneumonia), row H (M. fermentans incognitus). In FIG. 2C 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 .mu.g) 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. pneumonia, M. arginini, M. hominis, M. fermentans, M. genitalium, M. salivarium, U. urealyticum and A. laidlawii) and analyzed on Southern blots, being probed with .sup.32 P-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 .sup.32 P nick-translated psb-8.6 (3A) and psb-2.2 (3B), .sup.32 P end-labeled RS48 (3C), .sup.32 P labeled MI-H 3.3 (3D) and .sup.32 P end-labeled cDNA probe of E. coli ribosomal RNA (3E). Each lane contained 0.2 microgram of EcoRI enzyme pre-digested DNA from Acholeplasm 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 procaryotive mycoplasmas and those of Escherichia coli bacterium. The same blot which was consecutively probed with RS48 and MI-H 33 was reprobed with .sup.32 P-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 straining 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 pathogensis 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 concomittantly 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.sup.+ 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 .sup.32 P-labeled DNA derived from gradient banded M. fermentans incognitus. The insert of the phage clone is then recloned into the EcoRI site of Bluescript KS (M 13.sup.-) vector (Stratogene) 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) of monkeys 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 proteinase, 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 transfectants 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 DNAtransfecting 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&1) structures, L and R, in the element (see FIG. 5) (SEQ ID NO:3). L(.DELTA.G=-16.8 kcal/mol) is located exactly at the left terminus of the element, while R (.DELTA.G=-14.4 kcal/mol) is located very near the right terminus. Both of the potential s&1 structures are followed by a stretch of T residues pointing toward the interior of the element. These s&1 structures with T resides strongly resemble transcription terminators (Rosenberg and Court, Annu. Rev. Genet., 13 319 (1979), which would prevent transcription from the outside into the element (Syvanen, 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&1 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&1 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&1 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 antiphagocytosis in this pathogenic bacteria is known to be associated with Pep M5 protein (Fox, Bacteriol. 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 extracelluar 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 serial 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 .sup.32 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 mulitiple 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&1 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).

J. Detection of M. fermentans incognitus DNA by PCR

A polymerase chain reaction (PCR) assay to detect M. fermentans was designed on the basis of specific nucleotide (nt) sequences found at one terminus of the cloned incognitus strain of M. fermentans DNA psb-2.2 (SEQ ID NO:2). Primers (RS47 (SEQ ID NO:13) and RS49 (SEQ ID NO:14)) were chosen to produce an amplified DNA fragment of 160 bp. (See Examples 16 and 19.) The PCR assay detected very specifically the mycoplasmas of M. fermentans species but not other human or hon-human mycoplasmas, bacteria or eucaryotic cell DNA that we tested. However, this highly 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 incognitus strain of M. fermentans, from a strain (k7) previously islated 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 developmed 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 amply 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.times.10.sup.6 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 immunnodeficient 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 diseasedanimals. 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,Cchronic Fatigue Syndrome, Wegener's Disease, Sarcoidosis, respiratory distress syndrome, Kibuchi's disease, antoimmune 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, 21 (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 incognitus-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 incognitus 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 incognitus or M. fermentans can be used. A diagnostic kit for the detection of M. fermentans incognitus-specific or M. fermentans-specific antibodies can be prepared in a conventional manner using M. fermentans incognitus 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 incognitus 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 incognitus.

Antibodies against M. fermentans incognitus (or M. fermentans) can be produced in experimental animals such as mice, rabbits and goats, using standard techniques. Alternatively, monoclonal antibodies against M. fermentans incognitus (or other strains of M. fermentans) antigens can be prepared in a conventional manner. Homologous antibodies are useful for detecting antigens to M. fermentans incognitus 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 incognitus antigens in tissues of patients infected by the mycoplasma.

Rabbit antiserum has been prepared using M. fermentans incognitus. The antiserum positively immune stains brain and lymph node tissue from AIDS patients. To produce the antiserum, sucrose gradient banded M. fermentans incognitus 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 incognitus. This capability is useful for the isolation of M. fermentans incognitus from other tissues. For example, additional M.

fermentans incognitus 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 incognitus 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 incognitus from the infected cell lysate.

N. Vaccines

The M. fermentans incognitus pathogen, antigens of M. fermentans incognitus 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 incognitus (or other strains) or can be produced by conventional recombinant DNA techniques. The vaccines are prepared by usual procedures, such as by 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 incognitus pathogen is isolated from the infected cells grown in monolayers. M. fermentans incognitus is killed by known procedures or modifications thereof, e.g., by the addition of betapropiolactone, Formalin or acetylethyleneimine, by ultraviolat radiation, or by treatment with psoralen or psoralen derivatives and long-wavelength ultraviolet light. Alternatively, M. fermentans incognitus 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 incognitus 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 incognitus 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 incognitus can also be used as vaccines to induce the formation of protective antibodies or cell-mediated immunity to M. fermentans incognitus. It has been found that antigens of other mycoplasmas share many antigenic determinants with M. fermentans incognitus, but lack the pathogenicity of M. fermentans incognitus. One such mycoplasma which can then be used in a vaccine against M. fermentans incognitus is M. fermentans. Other mycoplasmas useful in vaccines against M. fermentans incognitus 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, quinalones

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 Cytocidal Effects in CD4.sup.+ Lymphocytes by M. fermentans incognitus.

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.

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 37.degree. 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 65.degree. C. for 30 minutes and at 37.degree. C. for ten hours. Phenol extraction (twice) followed by phenol/chloroform/isoamylalcohol (25:24:1) and chloroform/isoamylalcohol (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.times.10.sup.6 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 fibroblast, or salmon sperm were precipitated with calcium phosphate in each 60 mm Petri dish culture (containing about 5.times.10.sup.5 NIH/3T3 cells). The DNA precipitate was removed after cells were incubated at 37.degree. 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 recovered 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 the 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 .sup.32 P 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.times.10.sup.5 cells/ml) in square petri dishes. The cultures were incubated at 37.degree. C. in a 5% CO.sub.2 incubator for 48 to 72 hours. The culture slides were washed three times with cold PBS, air-dried and stored at 4.degree. 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.degree. C. in 2.5% glutaraldehyde in phosphate buffer and post-fixed with 1% OsO.sub.4. 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 incognitus 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.degree. 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 multicellular 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 incognitus 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 incognitus nucleocapsids within the nucleus is prominent. Numerous M. fermentans incognitus particles of different maturation stages were seen in the cytoplasm at 45,800X magnification. Most of the mature M. fermentans incognitus cells in the cytoplasm are lined up along the plasma membrane while others are free. The M. fermentans incognitus cells were roughly spherical enveloped particles of heterogenous sizes. The majority of mature M. fermentans incognitus cells were 140-280 nm, with an overall range of 100-900 nm. The intact M. fermentans incognitus 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 incognitus cell. Although the M. fermentans incognitus 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 incognitus cells (at 45,800X magnification).

To further confirm the ultrastructure and morphology of M. fermentans incognitus, the unsectioned M. fermentans incognitus were examined by pelleting M. fermentans incognitus 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 incognitus particles were briefly fixed with 0.5% Formalin to preserve the M. fermentans incognitus 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 101,800X 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 ug/ml) and the sample was digested at 56.degree. C. for 2 hrs. Before PCR analysis the digested samples were heated at 95.degree. 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 MgCl.sub.2, 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 uM, each dNTP at 250 uM 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.degree. C. for 35 sec, annealing of primers at 56.degree. C. for 45 sec and extension at 72.degree. 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.times. 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 (Bio-Rad, Richmond, Calif.) at 100 volts for 2 hrs., in 0.5.times. 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.times. SSC in 20 mM Tris-HCl (pH 7.5) and air dried for 10 min. Prehybridization was carried out in 30% formamide, 4.times. SSC, 5.times. Denhardt's, 20 mM Tris-HCl, (pH 7.5), 2 mM EDTA, 1% SDS and 350 ug/ml of denatured salmon sperm DNA at 30.degree. C. Hybridization was in the same mixture but contaiing the oligonucleotide probe RW006 (SEQ ID NO:17) (Wang et al., Abstr. Gen. Meet. Am. Soc. Microbiol. 1991, G-5. p. 134) which was 5'end labeled with .sup.32 P-ATP, and was conducted overnight at 30.degree. C. After hybridization the membrane was washed at 45.degree. C. in 2.times. 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 microgram of human placental DNA and lane o contained pUC18 DNA digested with MspI, 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 deteted 1 fg of DNA in all species (FIG. 7). Specificity of the reaction has also been examined by attempting to amply 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 _____ SPECIFICITY OF PCR FOR M. FERMENTANS USING UNIQUE SEQUENCES WITHIN THE IS-LIKE GENETIC ELEMENT Concentration of DNA Sources tested Positivity _____ Mycoplasmas M. fermentans ATCC 19989 1 fg + incognitus strain 1 fg + PG-18 1 fg + K-7 1 fg + MT-2 1 fg + and nine clinical isolates 1 fg + M. hominis (ACTCC 15488) 1 ng - M. orale (ATCC 23714) 1 ng - and one clinical isolate 1 ng - M. salivarium (ATCC 23064) 1 ng - and two calinical isolates 1 ng - M. buccale 1 ng - M. pneumoniae (ATCC 15531) 1 ng - M. genitalium (ATCC 33530) 1 ng - M. arginini (ATCC 23838) 1 ng - M. pirum 1 ng - M. alvi 1 ng - M. moatsii 1 ng - M. sualvi 1 ng - M. iowae 1 ng - M. arthritidis 1 ng - M. hyorhinis (ATCC 17981) 1 ng - Acholeplasma laidlawii (ATCC 23206) 1 ng - Ureaplasma urealyticum 1 ng - (ATCC 27618) Bacteria E. coli 1 ug - Streptococcus pneumoniae 1 ug - Clostridium perfringens 1 ug - Mouse NIH/3T3 1 ug - Spleen (Balb/c) 1 ug - Liver (Balb/c) 1 ug - Brain (Balb/c) 1 ug - Monkey Vero cells (ATCC CCL18) 1 ug - Spleen (green monkey) 1 ug - Liver (green monkey) 1 ug - Brain (green monkey) 1 ug - Human CCRF-cem (ATCC CCL119) 1 ug - Placenta (nl. delivery) 4X) 1 ug - PBMC (nl. donor) 50X 1 ug - _____

EXAMPLE 6

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

Urine was collected in sterile containers and concentrated 10-fold by centrifugation (3000.times.g for 15 min. at 4.degree. 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. (1989(a), 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 cm.sup.2 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 to a Zeta-Probe membrane by the Southern blot method. Each filter was prehybridized in 50% formamide, 4 x SSC, 5 x Denhardts', 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.degree. C. and hybridized with .sup.32 P nick-translated psb-2.2 DNA (Hu et al. (1990). Gene 93:67-72) at 42.degree. C. in the prehybridization solution as described, (Lo et al. (1989b), supra). After hybridization the blots were washed at 55.degree. C. in x 2 SSC, 0.5% SDS, 10 mM Tris-HCl (pH 7.5) for 120 min. with 4 changes and then washed at 50.degree. 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 modiifed 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 DNASs were digested with EcoRI (a lanes) or HindIII (b lanes), fractionated in an agarose gel and hybridized with .sup.32 P-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 grwon in mycoplasma culture from 3 urine sediments derived from 2 HIV positive individuals (Table 1). Five Ureaplams 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 _____ Isolation of Different Species of Mycoplasma and Ureaplasma urealyticum from Urine of HIV positive AIDS patients and HIV negative non-AIDS controls Source of Urine HIV Positive HIV Negative Species AIDS Patients Controls _____ M. fermentans 3/43.sup.a (7.0%).sup.b 0/50 (0%) M. fermentans 2/43 (4.7%) 1/50 (2.0%) U. urealyticum 5/43 (11.6%) 23/50 (46.0)% _____ .sup.a Number of isolates over number of samples cultured .sup.b 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.sup.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.sup.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 who 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 had died 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 periportal 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 periportal 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 pneumonitis. M-Ag and toluene 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 dermatities 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 hypothermia 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 Kaposi'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. Seroconversions 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 radioimmunoassay 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 infection 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 .sup.32 P. 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 apleen 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 immunoperioxidase straining 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-Ni-H.sub.2 O.sub.2 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 _____ Prevalence of Serum Antibodies to Sb51 Cells in AIDS Patients with Various Clinical Presentations Number Risk Group Positive for Male Antibodies Homo- Total to SB.sub.51 Subjects sexual Other Number Cells** _____

Patients with 23 1* 24 23 AIDS Kaposi's 8 8 8 sarcoma Opportunistic 5 1* 6 6 infection Kaposi's 10 10 9 sarcoma and opportunistic infections Normal 30 0** Controls _____ *Female, sexual partner of bisexual males. **Four nonAids control sera showed mild reactivity; all the other control sera did not elicit any reaction.

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.times.10.sup.7 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-cm.sup.2 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.