

## MYCOPLASMAS AND HIV INFECTION: FROM EPIDEMIOLOGY TO THEIR INTERACTION WITH IMMUNE CELLS

Catherine Brenner, Olivier Neyrolles, Alain Blanchard<sup>1</sup>

Institut Pasteur, Unité d'Oncologie Virale, Département SIDA et Rétrovirus, 28, rue du Dr. Roux, 75724 Paris Cedex 15, France

### TABLE OF CONTENTS

1. Abstract
2. Introduction
3. *In vitro* synergy between HIV and mycoplasma cell killing activity
4. Mycoplasma isolation from HIV-infected patients
5. Prevalence of mycoplasmas in HIV-infected patients
  - 5.1. Immuno-histochemistry evidence of mycoplasmas in tissues from AIDS patients
  - 5.2. PCR-based detection of mycoplasmas
  - 5.3. Seroprevalence of *M. penetrans* and *M. fermentans*
    - 5.3.1. *M. penetrans*
    - 5.3.2. *M. fermentans*
6. *M. fermentans* and *M. penetrans* cytopathogenicity
  - 6.1. Cell invasion
  - 6.2. Mycoplasma adherence to host cells
7. Mycoplasma interactions with the immune system
  - 7.1. Immune cell activators
  - 7.2. Antigenic surface variation and immune escape
  - 7.3. Molecular mimicry
8. Perspective
9. Acknowledgments
10. References

### 1. ABSTRACT

Mycoplasmas are possible HIV cofactors, contributing to the evolution of AIDS. Our knowledge about mycoplasma prevalence in HIV-infected subjects has considerably increased due the development of specific detection assays. A new mycoplasma, *Mycoplasma penetrans*, has been identified and has been shown to be associated with HIV infection, at least among individuals with homosexual practices. We and others investigated the properties of *M. fermentans* and *M. penetrans* concerning cell colonization, cell invasion and cytopathogenicity. The molecular components which are involved in the interaction between these bacteria

and immune cells are beginning to be identified and characterized. Membrane lipoproteins of these wall-less prokaryotes are key components in their interaction with B cells and surface capsular material may contribute to their defense from the host immune response.

### 2. INTRODUCTION

HIV-infection is associated with chronic but not maximal activation of the immune system (for review see 1). The persistence of virus replication throughout the course of HIV disease may contribute to the maintenance of this state of immune activation. The latter is directly linked to the rate of HIV replication because retroviral infection of new cells is facilitated by cellular activation and also HIV replication is initiated in cells latently infected with the virus by cell activation. There is now considerable evidence that the quantity of virus in plasma is predictive of the evolution of the disease (2).

HIV-1 replication *in vitro* is facilitated by T cell activation and therefore by several co-infectious

---

Received: 7/5/96; Accepted: 7/9/96

<sup>1</sup>To whom correspondence should be addressed, at the Institut Pasteur, Unité d'Oncologie Virale, Département SIDA et Rétrovirus, 28, rue du Dr. Roux, 75724 Paris Cedex 15, France, Tel #: (33-1)-40-61-31-31, Fax #: (33-1)-40-61-34-65, E-mail: ablancha@pasteur.fr

## Mycoplasmas and HIV Infection

agents, including mycobacteria, *Salmonella* spp. (3), *Leishmania donovani* (4). However, little is known about the ability of antigenic stimulation to augment HIV-1 replication *in vivo*. Recent studies have shown that vaccination of HIV-1 infected individuals is followed by a transient increase of HIV replication (5-7). Therefore, an antigenic stimulation produced by infectious micro-organisms is likely to produce a similar increase in HIV replication that may last as long as the infection persists. Silent mycoplasmal infections could act in this way as HIV cofactors (8). This putative role of mycoplasmas (trivial names for members of the class Mollicute) in the evolution of HIV disease is not restrictive and they may also facilitate the transmission of HIV.

Indeed, numerous studies have established that ulcerative or non-ulcerative sexually transmitted diseases (STDs) favor homosexual and heterosexual HIV transmission (9-11). Urogenital mycoplasmas may thus facilitate local HIV acquisition. Although heterosexual transmission is currently the major mode of HIV infection worldwide, the contribution to the epidemic of the mother-to-child transmission is increasing, notably in Africa and South-East Asia. Vertical transmission is possible *in utero* and through breastfeeding, but is believed to be mostly during delivery. The mother's HIV infection stage (CD4+ cell count and viral load) is highly predictive of the probability of transmission. Other factors have also been implicated but to a lesser degree. In particular, sexually transmitted diseases (STDs) were proposed to increase the transmission of HIV during pregnancy and the treatment of these STDs may reduce vertical transmission (12). Urogenital mycoplasmas, in particular *Ureaplasma urealyticum*, are a common cause of chorioamnionitis and are vertically transmitted (13, 14). They may thus facilitate vertical HIV-transmission.

### 3. *IN VITRO* SYNERGY BETWEEN HIV AND MYCOPLASMAS CELL KILLING ACTIVITY

Interaction between mycoplasmas and HIV was first suggested by *in vitro* observations following the addition of antibiotics to HIV infected-cell lines. Independently, Lemaître *et al.* (15) and Nozaki-Renard *et al.* (16) observed that the cytopathic effect of HIV was considerably reduced when tetracyclines or fluoroquinolone were added to mycoplasmas contaminated- and HIV-infected CEM cells. Lo *et al.* (17) and Lemaître *et al.* (18) demonstrated synergy increased cell death between HIV and the mycoplasmas *Mycoplasma fermentans*, *M. penetrans*, *M. pirum*, and *M. arginini*. This effect was observed when mycoplasmas were added to HIV-infected cell

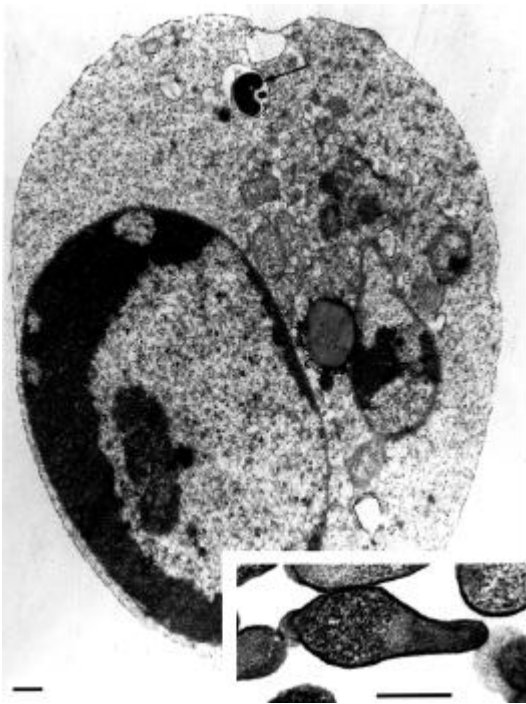
lines including lymphocytic CEM and the promonocytic U937 and THP1. Lemaître *et al.* (18) described this synergy for individual cell lysis rather than syncytia formation, whereas Lo *et al.* (17) reported the disappearance of HIV-associated syncytia formation in the presence of *M. fermentans*. In contrast, Chowdhury *et al.* (19) found increased HIV-infected MOLT-4 syncytia formation when extracts from the mollicute *Acholeplasma laidlawii* were added.

The molecular mechanism of this synergy is unknown. Quantitative analysis of electron micrographs of HIV-infected MOLT-4 cells indicates that HIV and *M. fermentans* tend to infect same cells ( $p < 0.001$ ) and that they attach to the same regions of the cell surface (in 90% of cases) (20). Possibly mycoplasmas have a direct effect on HIV attachment, entry and virion release. There is also evidence that mycoplasmas enhance HIV replication: *M. pneumoniae*, *M. genitalium*, and *M. fermentans* cause an increased rate in the viral replication (measured by RT and P24 assay) when added to infected human peripheral blood mononuclear cells (PBMCs) (21). Similarly, Chowdhury *et al.* (22) showed that heat-treated *A. laidlawii* extracts increased the HIV replication rate (by 45-fold) in both promonocytic J22HL-60 and U937 cells, and in monoblastic U1 cells. This stimulation involved a cellular signal transduction pathway implicating protein kinase C, as demonstrated by utilizing a specific inhibitor for this enzyme.

Oxidative stress results in a transactivation of HIV-LTR (23, 24), and various mycoplasma species (*M. fermentans*, *M. pirum*, *M. penetrans*, and *U. urealyticum*) enhance the HIV-LTR-dependent gene expression (25). Consequently increased HIV replication could, at least in part, be due to the production of reactive oxygen intermediates following cell infection with mycoplasmas (26, 27).

### 4. MYCOPLASMAS ISOLATION FROM HIV-INFECTED PATIENTS

Simultaneous with the publication of these *in vitro* observations, the isolation and detection of mycoplasmas in HIV-infected subjects was reported by various groups. *M. fermentans* was the first mycoplasma species reported to be invasive in AIDS patients. The first isolate, named *incognitus*, was identified as a strain of *M. fermentans* (28-31). In our laboratory, mycoplasma strains including *M. fermentans* (AOU) and *M. pirum* (BER) were isolated from blood of HIV seropositive patients (18). In another study, *M. fermentans* was isolated from the



**Figure 1:** Ultrastructural morphology of *M. penetrans*. A *M. penetrans* organism indicated by the arrow is shown within the cytoplasm, inside a vacuole of an lymphocyte experimentally infected with the mycoplasma. The insert shows a single *M. penetrans* cell from an *in vitro* culture; the thin section was treated with ruthenium red which reveals external layer of the capsule-like material. In both electron micrographs, the bar represents 300nm.

urine of two of 43 patients and seven were PCR-positive for this mycoplasma (32). The low sensitivity of culture as compared to PCR is a common characteristic of some mycoplasma species which are known to be extremely fastidious and therefore difficult to grow. This property is also exemplified by results from another study in which *M. fermentans*, identified using specific antiserum, could be cultured for one passage from several of urine samples collected from HIV-infected subjects, but subcultures failed to grow (33). Prior to these observations, *M. fermentans* was considered as a species rarely isolated from the human urogenital tract. It has been implicated in rheumatoid arthritis although the evidence is conflicting. Indeed recently published data indicate that the putative association with arthritic diseases remains under investigation (34).

*M. penetrans* was initially isolated from urine from six HIV seropositive male homosexuals (35, 36). To date, *M. penetrans* has only been isolated

from HIV-infected subjects although epidemiological studies (see below) have indicated its presence in other HIV-seronegative subjects. This novel mycoplasma has an elongated flask shape with a terminal structure as shown in Figure 1.

Under the electron microscope, two distinct compartments are observed: a densely packed tip-like structure and a broader body compartment with a more heterogeneous cytoplasm structure. A similar morphology has been described for another mycoplasma, *M. iowae*, a poultry pathogen. Comparative analysis of 16S rRNA genes indicates that *M. penetrans* and *M. iowae* are phylogenetically related (37).

### 5. PREVALENCE OF MYCOPLASMAS IN HIV-INFECTED SUBJECTS

It was difficult to evaluate the prevalence of these mycoplasmas (*M. fermentans* and *M. penetrans*) in HIV-infected subjects from the initial studies based on isolation procedures, because control groups were not always included and the number of recruited patients was limited. Various epidemiological studies were undertaken concomitantly with the development of detection methods including direct procedures such as immuno-histochemistry and PCR-based assays, or indirect methods such as enzyme-linked immunosorbent assay (ELISA) and Western blotting (WB) for anti-mycoplasma antibodies in patients' sera.

#### 5.1. Immuno-histochemistry evidence of mycoplasmas in tissues from AIDS patients

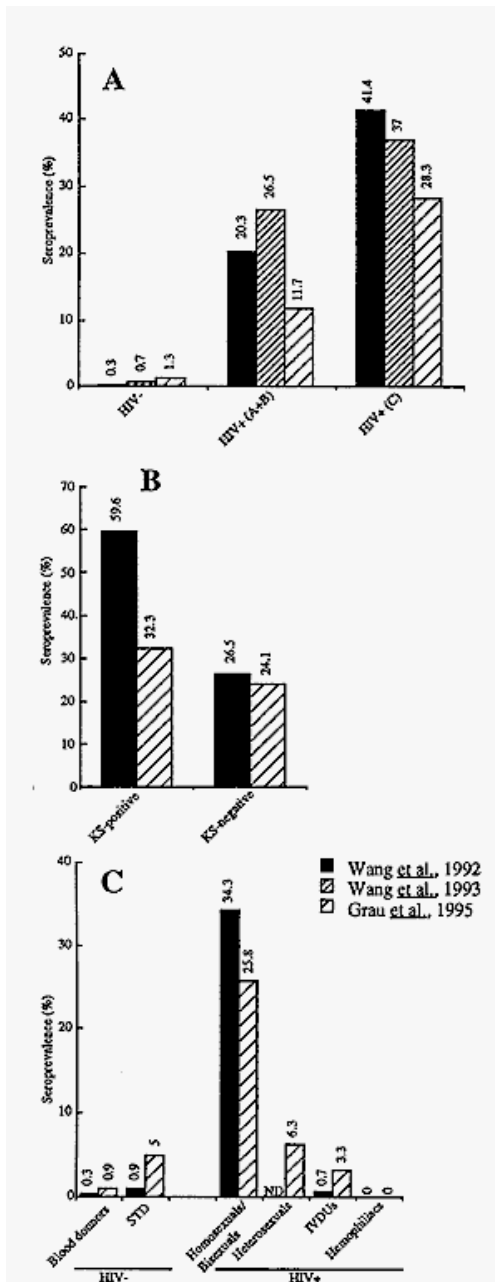
The presence of *M. fermentans* in AIDS patients was first reported in immuno-histochemistry studies (29, 38, 39) (see Table 1). The work of Lo *et al.* (29) indicated the frequent presence of *M. fermentans* in numerous tissues of patients dying with AIDS. Another group reported a much lower prevalence with only one patient out of 42 being positive at autopsy for this mycoplasma (38). Mycoplasma antigen was detected in spleen, liver and bone marrow of this patient whereas the brain, heart, testis and lymph nodes were negative. From these autopsy investigations, it is very difficult to evaluate the significance of the presence of *M. fermentans* in tissues of patients who died with an extensive destruction of their immune system.

*M. fermentans* has also been detected by immuno-histochemistry and electron microscopy in renal tissue taken at autopsy from a series of patients with AIDS (39). Twenty patients out of 203 had evidence of AIDS-associated nephropathy (AAN) and

## Mycoplasmas and HIV Infection

**Table 1:** *M. fermentans* identification and isolation in HIV-infected subjects.

Method	Identification or Isolation	Number of positives/site or tested site	References		
Immuno-histochemistry	23/24	Thymus 4/4 Liver 4/10 Lymph nodes 2/2 Spleen 6/8 Brain 6/8 Placenta 2/2	29		
	15/30 0/5 HIV-	Kidneys	39		
	1/42 0/3 HIV-	Spleen 1/26 Liver 1/31 Marrow 1/1	38		
	Serology	6/30; 2/50 HIV-	Serum	85	
		15%; 1.3% HIV-	Serum	41	
8/126		Serum	86		
14/180 (7.8%) 0/38 HIV-		Urine	33		
	11/39 (28%)	Serum	87		
PCR	7/10	Spleen 3/4 Lymph nodes 1/2 Liver 2/2 Brain 2/3 PBMCs 1/2 Kaposi's Sarcoma 1/1	28		
	6/55 (11%)	PBMCs	42		
	10/43 (23% and 5% isolation from culture) 0/50 HIV-	Urine	32		
	12/105 (11.4%) 6/105 (5.7%) 6/105 (5.7%) 2/105 (1.9%) 2/105 (1.9%)	Throat Genital Swab PBMCs PBMCs+Throat PBMCs+Genital Swab	43		
	12/117 (10%) 15/65 (23%) 4/55 (8%) 30% HIV-	PBMCs Throat Urine 3 sites	44		
	PBMCs:	Peripheral	Blood	Mononuclear	Cells



**Figure 2:** Comparison of results from epidemiological studies about the *M. penetrans* seroprevalence. The associations between *M. penetrans* seroprevalence and HIV infection, or development of Kaposi's sarcoma, or groups of HIV transmission are shown in panels A, B and C, respectively. HIV-/+ : HIV seronegative/ seropositive subjects. A, B and C are the stages of HIV infection. KS-positive/negative: patient positive/negative for Kaposi's sarcoma STD: patients attending clinic for sexually transmitted diseases IDUs: intravenous drug users.

*M. fermentans* was detected in all the 15 renal tissue samples available from these patients. Renal tissues from patients dying with AIDS without renal disease or without AIDS showed no *M. fermentans*-specific

**5.2. PCR-based detection of mycoplasmas**

A *M. fermentans*-specific PCR detection assay was developed by Wang *et al.* (41). Various studies using this assay report approximately the same *M. fermentans* prevalence, about 10%, in peripheral blood from HIV-infected patients (42-44) (table 1). Interestingly, *M. fermentans* was also detected in throat and genital specimens whereas *M. genitalium* and *M. pneumoniae* were not detected in these sites or in blood samples (44); (43). However, it seems that *M. fermentans* is not a common respiratory pathogen in AIDS patients because it was not detected in or cultured from bronchoalveolar lavages from HIV-infected subjects (45). The *M. fermentans* prevalence in blood from HIV seronegative individuals was evaluated in various control groups: *M. fermentans* was found in 9% of 73 seronegative patients that consulted for sexually-transmitted diseases (44), whereas Hawkins *et al.* (42) did not detect this mycoplasma in any of 26 blood donors.

These results are in apparent contradiction with those from another study which reports no evidence for mycoplasmas in peripheral blood mononuclear cells from HIV-infected subjects (46). However, these authors used a PCR assay with mycoplasma genus-specific primers for which the sensitivity of *M. fermentans* detection was not provided. Therefore, it is likely that these negative results were due to a lack of sensitivity rather than the absence of mycoplasma.

**5.3. Seroprevalence of *M. penetrans* and *M. fermentans***

**5.3.1. *M. penetrans*:**

Serological assays were developed, on an empirical basis, for the detection of *M. penetrans*-specific antibodies (47). The antigen preparation used is a Triton X-114 extract of mycoplasma cells, containing 2 major polypeptides of apparent molecular mass of 35 (P35) and 38 kDa (P38) (47). P35 and P38 are lipoproteins and the amino acid sequence deduced from p35 gene sequence contains a signal sequence (V<sub>21</sub>ATVPVIVSSC<sub>31</sub>) with a specific acylation site (48). The paper of Grau *et al.* (49) brought some confusion to the nomenclature by designating P35 and P38 as P38 and P42, respectively. In addition, the criteria for defining a *M. penetrans*-positive serum differs between the 2 groups: whereas a positive ELISA result is sufficient

## Mycoplasmas and HIV Infection

for Wang *et al.* (47), Grau *et al.* (49) propose that this must be confirmed by reactivity for P35.

The initial study of Wang *et al.* (47), as shown on Figure 2, established that 40% of AIDS patients and 20% of asymptomatic HIV infected patients were *M. penetrans* seropositive, whereas only 0.9% of STD patients and 0.3% of blood donors were *M. penetrans* seropositive. An association between *M. penetrans* and HIV infections was therefore suggested. However, others propose rather an association between this mycoplasma and homosexual practices (50). An epidemiological study by the same group indicated that HIV-infected male homosexuals whether suffering from AIDS or asymptomatic were much more frequently *M. penetrans* seropositive than other risk exposure categories (1% for intravenous drug users, and 0.6% among haemophiliacs) (51). The *M. penetrans* seroprevalence among French patients is lower: 18% of HIV-infected patients and 1.3% of HIV-seronegative patients were found to be *M. penetrans* seropositive (Figure 2). Both studies were consistent with an association between HIV and *M. penetrans* and suggest an association between homosexual behaviour and *M. penetrans*, and demonstrate that *M. penetrans* seroprevalence increases with progression of the disease. However, the two studies differ about the association between *M. penetrans* and Kaposi's sarcoma (KS): Wang *et al.* (51) found an association, whereas Grau *et al.* (49) did not.

A novel herpes virus, HHV8, has been detected in all KS lesions tested and its detection in the blood is predictive of KS development. This has changed our view of the genesis of the disease (52, 53). Whether *M. penetrans* also contributes to or facilitates KS development is unclear.

### 5.3.2. *M. fermentans*

There is little data available concerning the serological response to *M. fermentans*. Unlike for *M. penetrans*, no serological assay has been specifically developed for this species. Without indicating the method of detecting antibodies, it was reported that 15% of HIV-infected patients vs. 1.3% of HIV-seronegative individuals were *M. fermentans*-seropositive (51). Other serological studies based on growth or metabolic inhibition have given varied results, which are difficult to interpret (Table 1).

## 6. *M. Fermentans* AND *M. Penetrans* CYTOPATHOGENICITY

We will not review all the possible mechanisms of mycoplasma virulence which have

been discussed elsewhere (26). We will focus on cell invasion and attachment.

### 6.1. Cell invasion

Initial studies of the interaction between HIV and mycoplasmas were based on the view that the bacteria were exclusively extracellular parasites. However, the work of Lo *et al.* (28) caused several investigators to reevaluate this view and several mycoplasma species were subsequently found intracellularly. The demonstration of their capacity to survive and to replicate in the host cell cytoplasm was hampered by the fact that classical techniques used for the quantitative study of entry for other eubacteria such as *Shigella spp.* or *Listeria spp.* are not directly transposable to mycoplasmas. Indeed, the two classes of antibiotics (tetracyclines and fluoroquinolones) which are the most effective *in vitro* against mycoplasmas are known to penetrate the cytoplasm of eukaryotic cells, and cannot therefore be used for selective elimination of extracellular microorganisms. In addition, the standard method used for releasing intracellular bacteria from the eukaryotic cells involves Triton X-100. As mycoplasmas are sensitive to this neutral detergent, it is unsuitable for their isolation.

Taylor Robinson *et al.* (54) performed an electron microscopy study using a combination of ruthenium red staining and immuno-gold labeling to document *M. fermentans* (strains *incognitus* and PG18) invasion of Hela cell cultures. The mycoplasma was found in the cytoplasmic fraction or membrane-bound vacuoles of experimentally infected cells. These observations have been confirmed by other investigators (36, 55). This invasive ability could account at least in part for the cytopathic effects of this mycoplasma but it remains unclear if various mycoplasmas found in vacuoles result from microbial replication or from multiple cell invasion. Indeed, it has not been demonstrated that intracellular mycoplasmas are alive and can replicate. In another study in which the *M. fermentans* cytopathogenicity was evaluated for tracheal tissue *in vitro* and *in vivo*, there were large difference between the capacity of different strains of *M. fermentans* to induce ciliostasis and their cytopathic effect (56). *M. fermentans incognitus* was found to be more invasive than other strains. However it has been propagated much less than other strains. Therefore, the difference in cytopathogenicity might be due to loss of virulence during *in vitro* passages.

The invasive properties of *M. penetrans* were evidenced from its initial characterization, hence its name (36, 35). A novel technique was

## Mycoplasmas and HIV Infection

proposed to quantify *M. penetrans* cell invasion: a combination of gentamycin and Triton X-100 at low concentration kills extracellular mycoplasmas (8 log decrease) without affecting the intracellular microbes (57). Although it is reported that Triton X-100 treatment greatly potentiates gentamycin activity treatment, the choice of this antibiotic is surprising because *M. penetrans* has been shown to be resistant to gentamycin (58). However, using this method Andreev *et al.* (57) reported that *M. penetrans* internalization, but not cell attachment, was abolished by cytochalasin D, an inhibitor of actin polymerization, and significantly affected by tyrosine kinase inhibitors including staurosporin and genistein. Furthermore, *M. penetrans* cell entry was associated with the phosphorylation of a cell protein of 145 kDa, but not of the Hp90 protein, as for *E. coli* entry. *M. penetrans* cell entry has also been studied by confocal microscopy (59), which reveals that all mycoplasmas are internalized only 2 hours after infection, and *M. penetrans* attachment and entry induce large local rearrangements of the cellular cytoskeleton. This was confirmed by others who demonstrated that *M. penetrans* attachment modifies the distribution of tubulin,  $\alpha$ -actinin and aggregated phosphorylated cellular proteins (60).

### 6.2. Mycoplasma adherence to host cells

Both anti-*M. penetrans* antibodies and trypsin treatment inhibits bacterial attachment to HEp-2 cells, suggesting the involvement of microbial proteins (such as adhesins) in the cytoadherence process (60). Bacterial attachment is also strongly reduced by metaperiodate and neuraminidase treatment of mycoplasmas, implicating surface sugars containing sialic acid in bacterial cytoadherence, as suggested elsewhere (57). To try to identify the eukaryotic receptor for *M. penetrans* a cell-blotting technique was used (60). Biotin-labeled *M. penetrans* were found to bind fibronectin and this binding was not inhibited when mycoplasmas were preincubated with a 70 kDa fragment of fibronectin containing the heparin-gelatin binding domain, nor by the RGD peptide. The mycoplasma factors recognizing this putative receptor were isolated by affinity chromatography with immobilized fibronectin. A 65 kDa protein (P65) was thereby obtained. In addition, several other mycoplasma polypeptides (18 kDa, 28 kDa, 32 kDa, 36 kDa, 39 kDa, and 41 kDa) also bound HEp-2 cells on blots.

*M. penetrans* cell adhesion appears to be cell line-independent as it attaches to HEp-2, Hela, Wi38-VA13 and NCM/SM cells. This is consistent with the possibility that *M. penetrans* binds common

cell surface proteins, such as fibronectin, as has been shown for the syphilis spirochete and *Candida* spp.

The P36, P39, and P41 HEp2-binding proteins from *M. penetrans* may be lipid associated membrane proteins (LAMPS, 47). In particular, the lipoprotein P35 which can be extracted with Triton X-114 is a candidate for the P36 described by Giron *et al.*(60). The attachment of *M. penetrans* to eukaryotic cell seems to implicate both bacterial adhesins and surface sugars. This is not surprising as *M. penetrans* cells are surrounded by a polysaccharide capsule-like material that we have partly characterized (61).

## 7. MYCOPLASMA INTERACTIONS WITH THE IMMUNE SYSTEM

The level of immune activation is a key factor in HIV disease progression. Analysis of the mechanisms by which, and indeed whether, mycoplasmas modulate the host immune functions would therefore advance our understanding of their role, if any, as AIDS disease cofactors. Mycoplasma infections are generally chronic and the humoral arm of the immune host response includes in particular the production of antibodies against membrane lipoproteins. The persistence of these infections is probably due to mycoplasmas expressing two mechanisms: antigenic surface variation, a mechanism which would allow immune escape, and/or molecular mimicry of the host.

### 7.1. Immune cell activators

Numerous mycoplasmas, as whole organisms, are immune cells activators. They are mitogenic *in vitro* for lymphocytes, induce B-cell differentiation and trigger secretion of cytokines including interleukin-1 (IL-1), IL-2, IL-4, IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferons, and granulocyte macrophage-colony stimulating factor (GM-CSF). These results were obtained with cells from various host species including human, rat, mouse and guinea pig (for review and references see (62)).

Viable *M. penetrans* induces a complete cellular immune response, including proliferation, blastogenesis and expression of activation markers by T lymphocytes from healthy or HIV infected patients (63). Furthermore, heat-inactivated *M. fermentans* and *M. penetrans* retain some stimulating properties and various molecular components responsible for these effects have recently been or are being identified. Indeed, membrane extracts containing lipoproteins from these two mycoplasmas trigger

## Mycoplasmas and HIV Infection

cellular proliferation, secretion of cytokines, TNF- $\alpha$ , IL-1 and IL-6, and IgG and IgM secretion from mouse splenocytes (64), human myelomonocytic cell lines and monocytes (65-67). A mycoplasma extract highly enriched in lipoproteins can be prepared with Triton X-114. However free membrane lipids and capsule components are also co-extracted and therefore, the effects of such extracts cannot be exclusively attributed to lipoproteins unless appropriate control experiments are performed. A protein fraction from *M. fermentans* and *M. penetrans* kills non-differentiated myelomonocytic cells whereas no similar has been observed for similar extract from other mycoplasma species (65). This killing requires the presence of actinomycin D and resembles apoptosis. However, it differs from TNF-induced death and does not act via the cell surface receptor CD95/Fas/Apo-1. The physiological significance of this finding is unknown because the mycoplasmas by themselves have not been shown to induce apoptosis.

We have studied the molecular interactions between spiralin, a mycoplasma lipoprotein, and immune cells. Spiralin was chosen because of its availability in a highly pure form and free of detergent, in a micellar structure (68). Spiralin polyclonally stimulated B-cells from mouse splenocytes (69), similar to the B-cell activation by an LPS-independent pathway reported for various bacterial lipoproteins such as Braun's lipoprotein of *E. coli*, OspA and OspB of *Borrelia burgdorferi* or the *Treponema pallidum* lipoproteins (70-73). Mycoplasma lipoproteins are potent immunogens and are major components of the mycoplasmal membranes. This is consistent with the strong and specific humoral response that develops against them during natural infections (74, 51). Non-protein membrane components have also been shown to be involved in the mycoplasma-associated activation. They include *M. fermentans*-derived high-molecular-weight material (MDHM) and a recently identified *M. penetrans* glycolipid fraction (GLF). MDHM induces strong IL-6 secretion from murine and human macrophages, and a cytolysis in murine thymocytes but does not induce a proliferative response of murine splenic cells (75-77). GLF is a strong mitogenic and induces differentiation of B-cells, and release of IgG and IgM. It does not induce the secretion of pro-inflammatory cytokines (IL-1 and IL-6) from mouse splenocytes and thus differs from mycoplasmal lipoproteins (61).

### 7.2. Antigenic surface variation and immune escape

Various mycoplasma species undergo antigenic variation, a mechanism by which they alter

their surface architecture to evade immune recognition (78). However, the relationship between antigenic variation and mycoplasmal virulence is unknown (79).

The best documented case of mycoplasmal antigen variation is the *M. hyorhinis* variable lipoproteins (Vlps). Vlps genes corresponding genes encode proteins with characteristic C-terminal regions, the size of which varies due to insertion or deletion of intragenic repeats (80). Some Vlps have also been identified in *M. fermentans* (81), but there is no evidence for such proteins in *M. penetrans*. The P35 gene region contains direct and inverted repeated sequences which could allow antigenic variation through homologous recombination (48). Highly variable surface structure is a property common to several mycoplasma species (79, 82). Antigenic variability may explain the chronicity of mycoplasmal infection and thus further information on this issue would be valuable.

### 7.3. Molecular mimicry

Another mechanism often involved in immune system escape is molecular mimicry by structures of the parasite for its host. In contrast with its strong mitogenic properties toward mouse splenocytes, *M. penetrans* GLF had no effect on human PBMCs of 15 donors *in vitro*. This strongly suggests molecular mimicry of the GLF for the sugars exposed on human cell surfaces. Indeed, this is coherent with the lack of humoral response toward this compound in *M. penetrans*-infected patients, although GLF-immunized rabbits and macaques developed a humoral response (61). Possibly GLF, recently characterized as a capsule-like material, protects *M. penetrans* from host phagocytosis.

Another type of mycoplasmal molecular mimicry of eukaryote structures was proposed for the P1 adhesin of *M. pneumoniae* to evade the induction of adherence inhibiting antibodies, and this may influence the pathogenesis of the infection (83).

Altogether, there is various evidence for complex cellular and molecular activation of the immune system by mycoplasmas. Mycoplasma interactions with the immune cells involve proteins, lipoproteins and glycolipids, each having a particular effect on host cells to induce cellular and/or humoral responses. Moreover, lipoproteins were found in numerous mycoplasmal species, whereas MDHM and GLF have only been identified in *M. fermentans* and *M. penetrans*, respectively. Therefore, any conclusions about their influence on immune cells cannot be extended to all the mycoplasma species.



### 8. PERSPECTIVE

Other than the association between *M. fermentans* and AIDS-associated nephropathy, the pathogenic potential of this mycoplasma in AIDS patients is not defined. In patients not infected with HIV, there are reports indicating it is associated with a flu-like illness, with respiratory distress syndrome and/or systemic disease with multiple organ failure (17, 55, 84). In a few of the described cases, erythromycin treatment was ineffective whereas administration of doxycycline, at least in one patient, was followed by cure (17); these observations correspond to the antibiotic sensitivity of *M. fermentans* (resistant to erythromycin and sensitive to doxycycline) consistent with an etiologic role for the mycoplasma in these patients' diseases. Finally the pathogenic role of this mycoplasma in HIV-seropositive subjects and in patients with respiratory disease or with various types of arthritis is being investigated by several groups.

One of the major difficulties in evaluating the pathogenic role of *M. penetrans* in humans is that it cannot easily be recovered or cultured from clinical samples. Although it has been detected in a large number of patients, its presence is not associated with any particular disease or symptoms. Presumably *M. penetrans* like most mycoplasma species, can behave as a commensal. In view of its cytopathic properties, it would be surprising if *M. penetrans* were not also an opportunistic agent at least.

The putative involvement of *M. penetrans* and *M. fermentans* as cofactors of HIV disease is currently being tested by epidemiological longitudinal studies and using the SIV infected macaque model.

### 9. ACKNOWLEDGMENTS

We thank Marie-Christine Prévost and Emmanuelle Perret for skilled electron microscopy and Stéphane Ferris for technical assistance in our studies. We would like also to thank particularly Pr. Luc Montagnier for constant support. This work was supported by grants from the Agence Nationale de Recherche sur le SIDA (ANRS), the Centre National pour la Recherche Scientifique (CNRS) and the Pasteur Institute. Catherine Brenner and Olivier Neyrolles are recipients of fellowships from the Association Claude Bernard.

### 10. REFERENCES

1. A. Fauci: Multifactorial nature of human immunodeficiency virus disease: implications for therapy. *Science* 262, 1011 (1993)
2. J. Mellors, C. Rinaldo, P. Gupta, R.M. White, J. Todd & L. Kingsley: Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272, 1167 (1996)
3. S. Mizel, L. Kucera, S. Richardson, F. Ciacci & N. Iyer: Regulation of macrophage activation and human immunodeficiency virus production by invasive *Salmonella* strains. *Infect Immun* 63, 1820-1826 (1995)
4. R. Bernier, S. Turco, M. Olivier & M. Tremblay: Activation of human immunodeficiency virus type 1 in monocytoïd cells by the protozoan parasite *Leishmania donovani*. *J Virol* 69, 7282-5 (1995)
5. S. Strapans, B. Hamilton, S. Follansbee, T. Elbeik, P. Barbosa & R. Grant: Activation of virus replication after vaccination of HIV-1 infected individuals. *J Exp Med* 182, 1727-1737 (1995)
6. D. Ho: HIV-1 viremia and influenza. *Lancet* 339, 1549 (1992)
7. W. O'Brien, K. Grovit-Ferbas, A. Namazi, S. Ovcak-Derzic, H. Wang & J. Park: HIV type 1 replication can be increase in peripheral blood of seropositive patients after influenza vaccination. *Blood* 86, 1082-9 (1995)
8. A. Blanchard, L. Montagnier: AIDS-associated mycoplasmas. *Ann Rev Microbiol* 48, 687-712 (1994)
9. W.E. Stamm, H.H. Handsfield, A.M. Rompalo, A.L. Ashley, P.L. Roberts & L. Corey: The association between genital ulcer disease and acquisition of HIV infection in homosexual men. *JAMA* 260, 1429-33 (1988)
10. J.-K. Kreiss, R. Coombs, F. Plummer, K-K. Holmes, B. Nikora & W. Cameron: Isolation of HIV from genital ulcers in Nairobi prostitutes. *J Infect Dis* 160, 380-4 (1989)
11. F. Plummer, J. Simonsen, D. Cameron, J. Nddinya-Achola & J. Kreiss: Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. *J Infect Dis* 163, 233-9 (1991)

## Mycoplasmas and HIV Infection

12. C. Peckham & D. Gibb: Mother-to-child transmission of the human immunodeficiency virus. *N Engl J Med* 333, 298-302 (1995)
13. W. Andrews, S. Shah, R. Goldenberg, S. Cliver, J. Hauth & G. Cassell: Association of post-cesarean delivery endometritis with colonization of the chorioamnion. *Obstet Gynecol* 85, 509-14 (1995)
14. F. Grattard, B. Soleihac, B. de Barbeyrac, C. Bébéar, P. Seffert & B. Pozzetto: Epidemiologic and molecular investigations of genital mycoplasmas from women and neonates at delivery. *Pediatr Infect Dis J* 14, 853-8 (1995)
15. L. Lemaître, D. Guétard, Y. Héning, L. Montagnier & A. Zerial: Protective activity of tetracycline analogs against the cytopathic effect of the human immunodeficiency viruses in CEM cells. *Res Virol* 141, 5-16 (1990)
16. J. Nozaki-Renard, T. Iino, Y. Sato, Y. Marumoto, G. Ohta & M. Furusawa: A fluoroquinolone (DR-3355) protects human lymphocyte cell lines from HIV-1 induced cytotoxicity. *AIDS* 4, 1283-86 (1990)
17. S.-C. Lo, S. Tsai, J.R. Benish, J.W. Shih, D.J. Wear & D.M. Wong: Enhancement of HIV-1 cytopathic effects in C lymphocytes by the AIDS-associated mycoplasma. *Science* 251, 1074-76 (1991)
18. M. Lemaître, Y. Henin, F. Destouesse, C. Ferrieux, L. Montagnier & A. Blanchard: Role of mycoplasma infection in the cytopathic effect induced by human immunodeficiency virus type 1 in infected cell lines. *Infect Immun* 60, 742-748 (1992)
19. M. Chowdhury, T. Munakata, Y. Koyanagi, S. Kobayashi, S. Arai & N. Yamamoto: Mycoplasma can enhance HIV replication *in vitro*: a possible cofactor responsible for the progression of AIDS. *Biochem Biophys Res Commun* 170, 1365-70 (1990)
20. D.M. Phillips, R. Pearce-Pratt, X. Tan & V.R. Zacharopoulos: Association of mycoplasma with HIV-1 and HTLV-1 in human T lymphocytes. *AIDS Res Hum Retroviruses* 8, 1863-68 (1992)
21. Y. Sasaki, M. Honda, M. Makino & T. Sasaki: Mycoplasmas stimulate replication of human immunodeficiency virus type 1 through selective activation of CD4<sup>+</sup> T lymphocytes. *AIDS Res Hum Retroviruses* 9, 775-80 (1993)
22. M. Chowdhury, T. Munakata, Y. Koyanagi, S. Arai & N. Yamamoto: Mycoplasma stimulates HIV-1 expression from acutely- and dormant-infected promonocyte/monoblastoid cell lines. *Arch Virol* 139, 431-8 (1994)
23. R. Schreck, P. Rieber & P. Bauerle: Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF- $\kappa$ B transcription factor and HIV-1. *EMBO J* 10, 2247-58 (1991)
24. N. Israël, M.-A. Gougerot-Pocidallo, F. Aillet & J.-L. Virelizier: Influence of the redox status of lymphocytes and monocytes on HIV expression and immune functions. Evaluation *in vitro* of antioxidant molecules as potential anti-HIV therapy. *Oxidative stress, Cell Activation and Viral Infection* 301-10 (1994)
25. R. Nir-Paz, S. Israel, A. Honigman & I. Kahane: Mycoplasmas regulate HIV-LTR-dependent gene expression. *FEMS Microbiol Lett* 128, 63-8 (1995)
26. V. Tryon & J. Baseman: *Mycoplasmas: molecular biology and pathogenesis*, Eds: J. Maniloff, *et al.*, Am. Soc. Microbiol.: Washington, DC. 457-71 (1992)
27. J. Chochola, A. Strosberg & M. Stanislawski: Release of hydrogen peroxide from human T cell lines and normal lymphocytes co-infected with HIV-1 and mycoplasmas. *Free Rad Res* 23, 197-212 (1995)
28. S.-C. Lo, J.W.-K. Shih, N.Y. Yang, C.Y. Ou & Y.-H. Wang: A novel virus-like infectious agent in patients with AIDS. *Am J Trop Med Hyg* 40, 213-226 (1989a.)
29. S.-C. Lo, M.S. Dawson, D.M. Wong, I.P.B. Newton, M.A. Sonoda, W.F. Engler: Identification of *Mycoplasma incognitus* infection in patients with AIDS: an immunohistochemical, *in situ* hybridization and ultrastructural study. *Am J Trop Med. Hyg* 41, 601-616 (1989b.)
30. C. Saillard, P. Carle, J. Bové, C. Bébéar, S.-C. Lo & J.-K. Shih: Genetic and serologic relatedness between *Mycoplasma fermentans* strains and a mycoplasma recently identified in tissues of AIDS and non-AIDS patients. *Res Virol* 141, 385-95 (1990)
31. T. Sasaki, Y. Sasaki, M. Kita, K. Suzuki, H. Watanabe & M. Honda: Evidence that Lo's Mycoplasma (*Mycoplasma fermentans incognitus*) is not a unique strain among *Mycoplasma fermentans* strains. *J Clin Microbiol* 30, 2435-40 (1992)

## Mycoplasmas and HIV Infection

32. M.S. Dawson, M.M. Hayes, R.Y.-H. Wang, D. Armstrong, R.B. Kundsin & S.-C. Lo: Detection and isolation of *Mycoplasma fermentans* from urine of human immunodeficiency virus type-1-infected patients. *Arch Pathol Lab Med* 117, 511-514 (1993)
33. K. Chirgwin, M. Cummings, L. DeMeo, M. Murphy & M. McCormack: Identification of mycoplasmas in urine from persons infected with human immunodeficiency virus. *J Infect Dis* 17, S264-6 (1993)
34. T. Sohaeverbeke, C. Gilroy, C. Bébéar, J. Dehais & D. Taylor-Robinson: *Mycoplasma fermentans* in joints of patients with rheumatoid arthritis and other joint disorders. *Lancet* 347, 1418 (1996)
35. S.-C. Lo, M.M. Hayes, R.Y. Wang, P. Pierce, H. Kotani & J.W.-K. Shih: Newly discovered mycoplasma isolated from patients infected with HIV. *Lancet* 338, 1415-1416 (1991)
36. S.-C. Lo, M.M. Hayes, J.G. Tully, R.Y. Wang & H. Kotani: *Mycoplasma penetrans* sp. nov., from the urogenital tract of patients with AIDS. *Int J Syst Bacteriol* 42, 357-364 (1992)
37. N. Behbahani, A. Blanchard, G. Cassell & L. Montagnier: Phylogenetic analysis of *Mycoplasma penetrans*, isolated from HIV-infected patients. *FEMS Microbiol Lett* 109, 63-66 (1993)
38. R. Miller-Catchpole, M. Shattuck, P. Kandalaf, D. Variakojis, J. Anastasi & C. Abrahams: The incidence and distribution of *Mycoplasma fermentans* (*incognitus* strain) in the Chicago AIDS autopsy series: an immunohistochemical study. *Mod Pathol* 4, 481-6 (1991)
39. F.A. Bauer, D.J. Wear, P. Angritt & S.-C. Lo: *Mycoplasma fermentans* (*incognitus* strain) infection in the kidneys of patients with acquired immunodeficiency syndrome and associated nephropathy. *Hum Path* 22(1), 63-69 (1991)
40. J. Ainsworth, V. Katseni, S. Hourshid & S. Waldron: *Mycoplasma fermentans* and HIV-associated nephropathy. *J Infect* 29, 323-6 (1994)
41. R.-H. Wang, W. Hu, M. Dawson, S. JWK & S.-C. Lo: Selective detection of *Mycoplasma fermentans* by polymerase chain reaction and by using a nucleotide sequence within the insertion element-like sequence. *J Clin Microbiol* 30, 245-48 (1992)
42. R.E. Hawkins, L.S. Rickman, S.H. Vermund & M. Carl: Association of mycoplasma and human immunodeficiency virus infection: detection of amplified-*Mycoplasma fermentans* DNA in blood. *J Infect Dis* 165, 581-585 (1992)
43. C. Bébéar, B. de Barbeyrac, M.-T. Clerc, H. Renaudin & H. Fleury: Mycoplasmas in HIV-1 seropositive patients. *Lancet* 341, 758-59 (1993)
44. V. Katseni, C. Gilroy, B. Ryait, K. Ariyoshi & P. Bieniasz: *Mycoplasma fermentans* in individuals seropositive and seronegative for HIV-1. *Lancet* 341, 271-73 (1993)
45. L. Teel, M. Finelli & S. Johnson: Isolation of *Mycoplasma* species from bronchoalveolar lavages of patients positive and negative for human immunodeficiency virus. *J Clin Microbiol* 32, 1387-89 (1994)
46. F. van Kuppeveld, W. Melchers, J. Kissing, J. van der Logt & J. Galama: No evidence of mycoplasmas in peripheral blood mononuclear cell fraction of HIV-infected patients. *Infection* 22, 204-5 (1994)
47. R.-H. Wang, J.-K. Shih, T. Grandinetti, P. Pierce, M. Hayes, D.J. Wear: High frequency of antibodies to *Mycoplasma penetrans* in HIV-infected patients. *Lancet* 340, 1312-16 (1992)
48. S. Ferris, H. Watson, O. Neyrolles, L. Montagnier & A. Blanchard: Characterization of a major *Mycoplasma penetrans* lipoprotein and of its gene. *FEMS Microbiol Lett* 130, 313-20 (1995)
49. O. Grau, B. Slizewicz, P. Tuppin, V. Launay, E. Bourgeois & N. Sagot: Association of *Mycoplasma penetrans* with human immunodeficiency virus infection. *J Infect Dis* 172, 672-81 (1995)
50. D. Taylor-Robinson & J. Ainsworth: Antibodies to *Mycoplasma penetrans* in HIV-infected patients. *Lancet* 341, 557-58 (1993)
51. R. Wang, J. Shih, S. Weiss, T. Grandinetti, P. Pierce & M. Lange: *Mycoplasma penetrans* infection in male homosexuals with AIDS: high seroprevalence and association with Kaposi's sarcoma. *Clin Infect Dis* 17, 724-9 (1993)
52. Y. Chang, E. Cesarman, M. Pessin, F. Lee, J. Culpepper & D. Knowles: Identification of Herpes-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266, 1865-1869 (1994)

## Mycoplasmas and HIV Infection

53. D. Whithby, M. Howard, M. Tenant-Flowers, N. Brink, A. Copas & C. Boshoff: Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *The Lancet* 346, 799-802 (1995)
54. D. Taylor-Robinson, H. Davies, P. Sarathchandra & P. Furr: Intracellular location of mycoplasmas in cultured cells demonstrated by immunocytochemistry and electron microscopy. *Int J Exp Pathol* 72, 705-14 (1991)
55. S.-C. Lo, D.J. Wear, S. Green, P. Jones & J. Legier: Adult respiratory distress syndrome with or without systemic disease associated with infections due to *Mycoplasma fermentans*. *J Infect Dis* 17, S259-63 (1993)
56. C.-H. Stadtländer, H. Watson, J. Simecka & G. Cassell: Cytopathogenicity of *Mycoplasma fermentans* (including strain *incognitus*). *Clin Infect Dis* 17, S289-301 (1993)
57. J. Andreev, Z. Borovsky, I. Rosenshine & S. Rottem: Invasion of HeLa cells by *Mycoplasma penetrans* and the induction of tyrosine phosphorylation of a 145-kDa host cell protein. *FEMS Microbiol Lett* 132, 189-94 (1995)
58. M. Hayes, H. Foo, J. Timenetsky & S.-C. Lo: *In vitro* antibiotic susceptibility testing of clinical isolates of *Mycoplasma penetrans* from patients with AIDS. *Antimicrob Agents Chemother* 39, 1386-7 (1995)
59. J. Baseman, M. Lange, N. Criscimagna, J. Giron & C. Thomas: Interplay between mycoplasmas and host target cells. *Microb Path* 19, 105-16 (1995)
60. J. Giron, M. Lange & J. Baseman: Adherence, fibronectin binding, and induction of cytoskeleton reorganization in cultured human cells by *Mycoplasma penetrans*. *Infect Immun* 64, 197-208 (1996)
61. O. Neyrolles, C. Brenner, M. Prévost, T. Fontaine, L. Montagnier & A. Blanchard: Isolation and characterization of a novel capsule-like material from *Mycoplasma penetrans*. *in preparation*, (1996)
62. G.J. McGarrity, H. Kotani & G.H. Butler: Mycoplasmas and tissue culture cells. In: *Mycoplasmas, molecular biology and pathogenesis*, Ed. J. Ma, *et al.* Washington, DC: ASM Press. (1992)
63. Y. Sasaki, A. Blanchard, H.L. Watson, S. Garcia, A. Dulioust & L. Montagnier: *In vitro* influence of *M. penetrans* on activation of peripheral T-lymphocytes from healthy donors or HIV-infected individuals. *Infect Immun* 63, 3916-21 (1995)
64. S.H. Feng & S.-C. Lo: Induced mouse spleen B-cell proliferation and secretion of immunoglobulin by lipid-associated membrane proteins of *M. fermentans incognitus* and *M. penetrans*. *Infect Immun* 62, 3916-21 (1994)
65. G. Rawadi, S. Roman-Roman, M. Castedo, V. Dutilleul, S. Susin & M. Geuskens: Effects of *Mycoplasma fermentans* on the myelomonocytic lineage. *J Immunol* 156, 670-678 (1996)
66. D.A. Kostyal, G.H. Butler & D.H. Beezhold: A 48-kilodalton *Mycoplasma fermentans* membrane protein induces cytokine secretion by human monocytes. *Infect Immun* 62, 3793-800 (1994)
67. G. Rawadi & S. Roman-Roman: Mycoplasma membrane lipoproteins induce proinflammatory cytokines by a mechanism distinct from that of lipopolysaccharide. *Infect Immun* 64, 637-643 (1996)
68. H. Wroblewski, S. Nyström, A. Blanchard & A. Wieslander: Topology and acylation of spiralin. *J Bacteriol* 171, 5039-5047 (1989)
69. C. Brenner, O. Neyrolles, M. Le Hennaf, H. Wroblewski, L. Montagnier & A. Blanchard: Respective roles of mycoplasma lipoproteins and capsule-like material in the *in vitro* interactions with immune cells. *In preparation*, (1996)
70. W. Bessler, M. Cox, A. Lex, B. Suhr, K.-H. Wiesmuller & G. Jung: Synthetic lipopeptide analogues of bacterial lipoprotein are potent polyclonal activator for murine B lymphocytes. *J Immunol* 135, 1900 (1985)
71. Y. Ma & J. Weiss: *Borrelia burgdorferi* outer surface lipoproteins OspA and OspB possess B-cell mitogenic and cytokine stimulatory properties. *Infect Immun* 61, 3843 (1993)
72. J. Radolf, L. Arndt, D. Akins, L. Curetty, M. Levi & Y. Shen: *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins activate monocytes/macrophages. *J Immunol* 154, 2866 (1995)
73. B. Kleine, R. Sprenger, C. Martinez-Alonzo & W. Bessler: Polyclonal B-cell activation by synthetic analogue of bacterial lipoprotein is functionally

## Mycoplasmas and HIV Infection

different from activation by lipopolysaccharide. *Immunology* 61, 29 (1987)

74. G. Jan, C. Fontenelle, M. Le Hénaff & H. Wroblewski: Acylation and immunological properties of *Mycoplasma gallisepticum* membranes proteins. *Res Microbiol* 146, 739-750 (1995)

75. P.F. Muhlradt & U. Schade: MDHM, a macrophage-stimulatory product of *Mycoplasma fermentans*, leads to *in vitro* interleukin-1 (IL-1), IL-6, tumor necrosis factor, and prostaglandin production and is pyrogenic in rabbits. *Infect Immun* 59, 3969-74 (1991)

76. P.F. Muhlradt, H. Quentmeier & E. Schmitt: Involvement of interleukin-1 (IL-1), IL-6, IL-2, and IL-4 in generation of cytolytic T cells from thymocytes stimulated by a *Mycoplasma fermentans*-derived product. *Infect Immun*. 59, 3962-3968 (1991)

77. P.F. Muhlradt & M. Frisch: Purification and partial biochemical characterization of a *Mycoplasma fermentans*-derived substance that activates macrophages to release nitric oxide, tumor necrosis factor, and interleukin-6. *Infect Immun* 62, 3801-07 (1994)

78. K.S. Wise: Adaptative surface variation in mycoplasmas. *Trends Microbiol* 1, 59-63 (1993)

79. H.L. Watson, X. Zheng & G.H. Cassell: Structural variations and phenotypic switching of mycoplasmal antigens. *Clin Infect Dis* 17, S183-6 (1993)

80. D. Yogev, R. Watson-McKown, R. Rosengarten, J. Im & K.S. Wise: Increased structural and combinatorial diversity in an extended family of genes encoding Vlp surface proteins of *Mycoplasma hyorhinis*. *J Bacteriol* 177, 5636-43 (1995)

81. K.S. Wise, M.F. Kim, P.M. Theiss & S.-C. Lo: A family of strain-variant surface lipoproteins of *Mycoplasma fermentans*. *Infect Immun* 3327-33 (1993)

82. X. Zheng, L. Teng, H. Watson, J. Glass, A. Blanchard & G. Cassell. Small repeating units within the *Ureaplasma urealyticum* MB antigen gene encode serovar specificity and are associated with antigen size variation. *Infect Immun* 63, 891-898 (1995)

83. E. Jacobs, A. Bartl, K. Oberle & E. Schiltz: Molecular mimicry by *Mycoplasma pneumoniae* to

evade the induction of adherence inhibiting antibodies. *J Med Microbiol* 43, 422-9 (1995)

84. W.R. Macon, S.-C. Lo, B. Poiesz, D. Montefiori, M. Dawson & T. Mason: Acquired immunodeficiency syndrome-like illness associated with systemic *Mycoplasma fermentans* infection in a human immunodeficiency virus-negative homosexual man. *Hum Pathol* 24, 554-558 (1993)

85. K. Hakkarainen, E. Jansson, A. Ranki, S.L. Valle & K.J.E. Krohn: Serological responses to mycoplasmas in HIV-infected and non-infected individuals. *AIDS* 6, 1287-92 (1992)

86. J.G. Tully, J.W.-K. Shih, R.H.-Y Wang, D.L. Rose & S.-C. Lo: Titers of antibody to mycoplasma in sera of patients infected with human immunodeficiency virus. *Clin Infect Dis* 17, 254-8 (1993)

87. S. Horowitz, J. Horowitz, E. Jacobs, E. Fuchs, B. Rager-Zisman & M. Alkan: Antibodies to *Mycoplasma fermentans* in HIV-seropositive and seronegative patients. *IOM Letters* 3, 354-5 (1994)