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Role of Mycoplasma Infection in the Cytopathic Effect Induced by Human Immunodeficiency Virus Type 1 in Infected Cell Lines

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In addition to previously reported tetracycline analogs, other antibiotics known for antimycoplasmal activities inhibited the cytopathic effect in CEM cl13 cells infected with human immunodeficiency virus type 1 (HIV-1) or HIV-2 but were unable to block virus replication. A contaminating mycoplasma was isolated from our CEM cl13 cells and identified as a strain of *Mycoplasma fermentans*. Following infection of lymphoblastoid (CEM) or promonocytic (U937 and THP1) cell lines with HIV-1, cytopathic effect was observed only in association with mycoplasmal contamination. Moreover, HIV-1 infection of U937 cells after experimental inoculation with a human isolate of *M. fermentans* led to pronounced cell killing. We have verified that this effect is not merely an artifact caused by arginine and/or glucose depletion in the cell culture medium. These results confirm that mollicutes, in particular *M. fermentans*, are able to act synergistically with HIV-1 to kill infected cells in some in vitro systems.

AIDS is characterized primarily by a profound alteration in the function and number of T4 lymphocytes, resulting directly or indirectly from infection with human immunodeficiency virus type 1 (HIV-1) or HIV-2 (2, 7). Several hypotheses were proposed to explain this specific cell killing associated with HIV-1 infection (9, 10), but none of them is sufficient to completely explain the extensive in vivo T4 cell destruction. Previously we demonstrated that tetracycline treatment of the HIV(LAV_{Bru})-infected CEM cl13 cell line prevented cell killing but not virus replication (12). A similar observation was made with another antibiotic, fluoroquinolone DR-3355 (27). Thus, it is possible that a microorganism could play, at least in vitro, the role of cofactor in HIV-induced cell lysis.

One likely microbial candidate could be a mycoplasma (class *Mollicutes*). Mycoplasmas are common contaminants of cell cultures. In addition, Lo and coworkers reported the isolation of an infectious microorganism, first called virus-like infectious agent (VLIA), that could be detected in patients with AIDS (15, 17). This microorganism seems to be pathogenic by itself in patients who do not have AIDS (14) and to cause fatal systemic infection in primates (19). VLIA was recognized as a mycoplasma, tentatively named *Mycoplasma incognitus* (16), and finally identified as a strain of *M. fermentans* (35). More recently, Lo et al. demonstrated that this particular mollicute enhances the cytocidal effect of HIV-1 in the A3.01 T lymphocytic cell line (18).

Our present results not only confirm that *M. fermentans* is effectively able to enhance the cytopathic effect (CPE) of HIV-1 in CEM cells but also extend our findings to U937 and THP1, promonocytic cells associated with an unidentified mollicute that is microbiologically distinguishable from *M. fermentans*. However, similar results were not obtained in HIV-infected H9 cells. In addition, we have shown that the mycoplasma-induced enhancement of cytopathicity is not a trivial consequence of arginine and glucose depletion in the culture medium.

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MATERIALS AND METHODS

Cells and viruses. Cell lines CEM (cl11 or cl13) and H9 are clones of a human T-lymphoblastoid CCRF-CEM cell line (ATCC CCL119) and T-lymphoma HuT78 cell line (ATCC TIB162), respectively, selected for their ability to efficiently replicate HIV. U937 (ATCC CRL1593) is a human promonocytic cell line, and THP1 (ATCC TIB202) is a human monocytic leukemia cell line. Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 IU of penicillin per ml, and 100 μg of streptomycin per ml. The antibiotics tested were mycoplasma removal agent, pefloxacin (Peflacin), minocycline (Mynocin), doxycycline (Vibramycin), clindamycin (Dalacin), erythromycin (Erythrocin), and chloramphenicol (Tifomycin or Salnicol). Mycoplasma removal agent is a quinolone of unknown structure supplied by Flow Laboratories (McLean, Va.). The other antibiotics were purchased from Sigma (St. Louis, Mo.) or obtained from the central pharmacy of the Rhône-Poulenc Rorer Research Center (CRVA, Vitry-Alfortville, France). Culture assays were performed as previously described (12, 36) in microtitration plates (96 wells) or in 24-well plates, as indicated.

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. In 50-ml tubes, 33 ml of blood was layered on top of 17 ml of Ficoll-containing lymphocyte separation medium (MSL; Eurobio, Paris, France). The tubes were centrifuged for 30 min at 400 × *g* at 4°C. Then, PBMC were collected and washed in complete culture medium. After trypan blue dye exclusion evaluation of the number of living cells, the PBMC were used immediately or placed in cryotubes (5×10^7 cells per tube) and frozen in liquid nitrogen. They were cultivated in supplemented RPMI 1640 medium containing interleukin-2 (10 U/ml; Lymphocult, Biotest).

The LAV_{Bru} strain of HIV-1 was used throughout this study. Virus stocks were obtained from Diagnostic Pasteur

(Marnes-la-Coquette, France) as a mycoplasma-free CEM cl11 cell supernatant. This supernatant was filtered through 0.45-μm filters, aliquoted, and stored at −80°C. When necessary, HIV was concentrated by centrifugation of the infectious supernatant (140,000 × *g*, 1 h, 4°C) and resuspension of the virus pellet in phosphate-buffered saline (PBS). The initial reverse transcriptase (RT) activity of this suspension was 16 × 10^6 cpm/ml when measured as described below.

Measurement of HIV replication and CPE. Briefly, 125 μl of cell suspension (8 × 10^4 cells per ml in supplemented RPMI 1640 medium) was incubated in microtitration plates for 1 h with 25 μl of the antibiotics (dissolved in 1% dimethylformamide–99% PBS) or PBS alone. The cultures were then infected by the addition of 100 μl of HIV-1, containing 50 to 250 times the minimal dose necessary to infect a CEM cell culture. Mock-infected cells received 100 μl of supplemented RPMI medium. Cells were incubated at 37°C in a 5% CO_2 incubator. At day 7 after HIV infection and then every 3 or 4 days, supernatant (60 μl) was removed for the RT assay (as described below) and 40 μl of the cell suspension was transferred to another microplate containing 210 μl of fresh medium supplemented with the antibiotic to be tested. The remaining cells in 100 μl were used for the cell viability assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) (29, 36). Viability was calculated by using the formula: % viable cells = [(A_{540} of HIV-infected + treated cells)/(A_{540} of mock-infected + treated cells)] × 100.

The cytocidal effect of mycoplasmas on HIV-infected cells was studied in 24-well microtiter plates. In this case, 2 ml of cell suspension (8 × 10^4 cells per ml) was infected with HIV-1 (same dose as above) in 500 μl of supplemented RPMI 1640. Mock-infected cells received only supplemented RPMI. An aliquot of cell culture supernatant was removed every 3 or 4 days to measure RT activity, p24 antigen concentration, and cell viability. The remaining cells were diluted to continue the incubation.

In vitro RT assay. As a marker of HIV replication, RT activity in cell supernatants was determined as described previously (36). Briefly, 50 μl of supernatant was incubated for 1 h at 37°C with 50 μl of RT reaction cocktail, containing 50 mM Tris-HCl (pH 7.8), 50 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 0.05% Triton X-100, 0.5 mM EGTA (ethylene glycol tetraacetic acid), poly(rA)-oligo(dT) (0.5 A_{260}/ml), and [^{3}H]TTP (0.11 MBq [1 μM]). The polynucleotides were then precipitated with 20 μl of 60% trichloroacetic acid in 120 mM $Na_4P_2O_7$, and the samples were filtered on glass fiber filters with a Skatron cell harvester (Skatron Instruments Inc., Sterling, Va.). The filters were dried, and radioactivity was counted in a beta scintillation counter (LKB, Uppsala, Sweden).

Virus replication was measured in parallel by the determination of p24 antigen levels in culture supernatants with an enzyme immunoassay kit (Du Pont, Wilmington, Del.).

Mycoplasma isolation, detection, and characterization. A clinical mycoplasma isolate was obtained from experiments that were originally designed to recover clinical HIV isolates from the blood of HIV-seropositive individuals. PBMC (6 × 10^6 cells) from HIV-seropositive patients were prepared by Ficoll centrifugation as described above and cultivated for 3 days in 6 ml of supplemented RPMI 1640. These cells were then cocultivated with 3 × 10^6 PBMC from a healthy HIV-seronegative donor that were previously activated for 3 days with 0.1% phytohemagglutinin (Difco Laboratories, Detroit, Mich.) in supplemented interleukin-2-containing RPMI 1640. Every 3 or 4 days, cells were evaluated for viability (trypan blue dye exclusion), virus production was measured (RT assay and p24 antigen detection), and the culture medium was renewed. After 11 days of coculture, supernatants from these samples were mixed with SP-4 mycoplasma medium (38) (1:9, vol/vol), incubated at 37°C, and checked daily for mycoplasma growth.

The *M. fermentans* strain AOU that was isolated during this study and the *M. pirum* strain BER (25) are available from the Collection of the Institut Pasteur (Paris, France). Mycoplasma contamination or experimental infection of cell lines, PBMC, HIV-1 stocks, and fetal calf serum was routinely tested by four different methods: culture in SP-4 mycoplasma medium (38), fluorescence staining with 4′,6-diamine-2′-phenylindole (DAPI) after coculture (3 and 6 days) with 3T6 indicator cells (32), detection of adenosine phosphorylase in cell culture supernatants (5), and microbiological cultures (1). In order to avoid cross-contamination of cell cultures, manipulations were performed essentially by the method of McGarrity et al. (21). Moreover, contaminated and noncontaminated biological materials were manipulated in separate laminar flow hoods and incubated in dedicated incubators.

Identification and characterization of mycoplasmas were performed by the serological and molecular approaches described by others (35). *M. fermentans*-specific antiserum was kindly provided by J. G. Tully (National Institute of Allergy and Infectious Diseases, Frederick, Md.).

Preparation of mycoplasma extracts. Mycoplasma extracts were prepared from three strains (*M. fermentans* AOU, *M. pirum* BER, and *M. hominis* type strain PG21). Mycoplasma cells were recovered from broth culture by centrifugation (12,000 × *g*, 15 min, 4°C) and washed twice in PBS. Cell lysis was achieved by sonication (four times for 30 s each at 0°C) of a mycoplasma suspension containing 5 × 10^5 color-changing units (CCU) per ml in PBS. The crude cytoplasmic fraction was separated from the membrane fraction by centrifugation (180,000 × *g*, 1 h, 4°C), and the cytoplasmic fraction was collected. The effect of mycoplasma extracts on RT activity was measured by incubation of these extracts (1 or 5 μl) with concentrated virus (1 or 5 μl) and RT reaction cocktail (50 μl). The final volume was adjusted to 100 μl with PBS, and the mixture was incubated for 1 h at 37°C. The enzymatic reaction was then stopped by the addition of 20 μl of 60% trichloroacetic acid in 120 mM $Na_4P_2O_7$. The precipitate was filtered and counted as described above.

PCR analysis of mycoplasma strains. Mycoplasma lysates were prepared as described previously (4), and DNA amplification was performed by the method of Saiki et al. (34). The oligonucleotides that were used as primers for the polymerase chain reaction (PCR) were 5′-GAATTCTTTAAT TGAGTTGCTC-3′ and 5′-AACCCCTTTCCAAAAAGTC CGG-3′. These primers were chosen according to a nucleotidic sequence that was reported to be specific for *M. fermentans* (*M. incognitus*) (17). DNA was amplified for 35 cycles at 95°C for 20 s, 58°C for 1 min, and 72°C for 1 min. The products of the reaction were resolved in a composite gel of 2% NuSieve and 1% Seakem agaroses (FMC Corp., Rockland, Maine) in Tris-borate buffer and stained in ethidium bromide solution.

RESULTS

Antibiotic effects on HIV-associated CPE. To confirm and extend our previous results with tetracycline analogs (12), we investigated the effect of several other antibiotics on

CEM cl13 viability after HIV-1 infection. Syncytia were observed in cultures between 4 and 10 days after HIV-1 infection. After this time, cytocidal effects were not seen with effective doses of antibiotics in the cultures, although infected cells continued to produce HIV-1 (Fig. 1). In contrast, there was substantial cell death in HIV-infected cultures supplemented with too little or inactive antibiotic. The most active compound for providing cell viability protection was mycoplasma removal agent, a quinolone of unknown structure (Fig. 1A). This compound reduced by 50% the CPE associated with HIV-1 replication at a dose of 0.001 μg/ml (50% effective concentration [EC_{50}]). Pefloxacin, another quinolone antibiotic, displayed an EC_{50} of 0.03 μg/ml (Fig. 1B). For minocycline (Fig. 1C) and doxycycline (not shown), the two most active tetracycline analogs, the EC_{50} was 0.5 to 1 μg/ml. Clindamycin (Fig. 1D) was active only above 3 μg/ml. Erythromycin (Fig. 1E) and chloramphenicol (Fig. 1F) were not able to provide complete protection at nontoxic doses. All compounds were toxic for noninfected cells at doses higher than those reported in Fig. 1.

Throughout these experiments, AZT (3′-azido-2′,3′-dideoxythymidine), added at a final concentration of 1 μM, inhibited both cell killing and viral replication, as expected for this antiviral agent (not shown). By contrast, none of the tested antibiotics active in our assays has antiviral activity (i.e., inhibition of RT activity or p24 antigen production).

Mycoplasma detection and identification. As the antibiotics in the previous experiments are active against mycoplasmas, we examined the CEM cell line for mycoplasma contamination. Using DAPI staining with the 3T6 indicator cell line (32), measurement of adenosine phosphorylase activity (5), and microbiological techniques (1), we confirmed that our particular clone of CEM cells was mycoplasma contaminated. In addition, the mollicute was isolated in SP-4 medium. This mollicute was able to hydrolyze arginine and ferment glucose, and its growth was inhibited with *M. fermentans*-specific antiserum. When analyzed by PCR with specific oligonucleotide primers for *M. fermentans* (*M. incognitus*), the DNA from this strain produced a specific amplified product (168 bp, lower band; Fig. 2, lane 2). As described previously for the strain of *M. fermentans* called *incognitus* (17), another slower-migrating band (about 185 bp) was also obtained. Although no explanation has yet been provided for the amplification of two DNA fragments, one can suggest that this is due to the existence of multiple copies (that may be heterogeneous) of this DNA sequence within the genome of *M. fermentans* (17). The CEM cell line was therefore contaminated with *M. fermentans*, which is a common contaminant of cell cultures. Indeed, about 5% of the mollicutes that contaminate cell cultures were reported to belong to this species (22).

A mycoplasma was similarly isolated from U937 cells. This mollicute hydrolyzed arginine but did not ferment glucose, indicating that it is not a member of the species *M. fermentans*. In addition, PCR amplification of DNA from this mycoplasma with the *M. fermentans*-specific primers described above was not successful (Fig. 2). However, only some strains of *M. fermentans* (K7, AOU, and the one isolated from CEM) could be amplified with these primers. The type strain PG18 of the same species was weakly positive. Controls with primers specific for *M. pneumoniae* (3) and *Ureaplasma urealyticum* (4) were also negative (not shown).

Mycoplasma reduction of RT activity. The enhancement of RT activity by treatment of HIV-1-infected cells with antibiotics could be explained by the reduction of mycoplasma products interfering with the in vitro RT assay, as reported in two recent articles (18, 39). To examine this possibility, a concentrated HIV-1 suspension was incubated with crude cytoplasmic extracts of mycoplasmas.

The effect of culture medium on the incorporation of tritiated TTP in the RT assay was examined. HIV-1 (25 μl) was mixed with 25 μl of PBS, RPMI 1640, or SP-4 medium and assayed for RT. The relative radioactivity obtained in the three reactions was 100, 62, and 14%, respectively. Therefore, subsequent mycoplasma extracts were prepared in PBS.

Extracts from only some mycoplasmas inhibited the RT assay. As shown in Table 1, the extract from *M. hominis* did not inhibit the RT reaction. On the other hand, extracts from *M. fermentans* and *M. pirum* inhibited the RT reaction. The addition of 5 μl of extracts from these two species inhibited 99% of the RT activity. Preincubation of the *M. fermentans* extract for 1 h at 60°C partly abolished this inhibition (only 40% inhibition compared with 99%, as reported in Table 1).

Mycoplasma contamination and HIV-induced CPE in various cell lines. To determine whether HIV-induced CPE in non-T-lymphocytic cell lines can also be dependent on the presence of mycoplasmas, we chose monocytic (THP1 and U937) cell lines that were contaminated or not with mycoplasmas. The selected cell lines were infected with HIV-1 cell supernatant assessed to be mycoplasma-free. The results, summarized in Table 2, confirmed that, in these in vitro systems, CPE and extensive culture destruction (more than 50% cell lysis) were observed only when HIV-1 infection was associated with mycoplasma contamination. When mycoplasma-free cells were infected with HIV-1, only a slight and transient decrease in viability (less than 20%) was measured.

However, we also observed that infection of H9 cells with HIV-1 (strain LAV_{Bru}) did not produce CPE and that experimental contamination with *M. fermentans* did not change this feature. In a similar preliminary experiment with normal PBMC, no enhancement of HIV-associated CPE was obtained in the presence of added *M. fermentans* (data not shown).

Isolation of *M. fermentans* from cultured PBMC from an HIV-seropositive patient. A mycoplasma was isolated from an 11-day-old culture of PBMC from an HIV-seropositive and asymptomatic individual. SP-4 medium was inoculated with the supernatant of this culture, and within 6 days, mycoplasma growth was indicated (color shift from red to yellow). However, no mycoplasma was recovered from six similar cultures of PBMC from other HIV-seropositive patients that were processed at the same time. The strain isolated (AOU) hydrolyzed arginine and fermented glucose and was identified as a strain of *M. fermentans* by growth inhibition with *M. fermentans*-specific antiserum. In addition, specific amplified products were obtained by PCR analysis with *M. fermentans*-specific primers (Fig. 2, lane 4).

Influence of mycoplasma infection on CPE induced by HIV-1. We tested the ability of *M. fermentans* AOU to act synergistically with HIV-1 to produce cell lysis. For that purpose, mycoplasma-free U937 cells were inoculated with 5×10^5 CCU of strain AOU. Addition of the mycoplasma induced a cytostatic effect on days 4 and 7 postinoculation with no gross cytotoxicity (fewer cells than in the control mycoplasma-free U937 but without killed cells, as measured by trypan blue dye exclusion). After 14 days, mycoplasmas could still be recovered from the experimentally infected cell cultures by using SP-4 medium, while uninfected cells re-

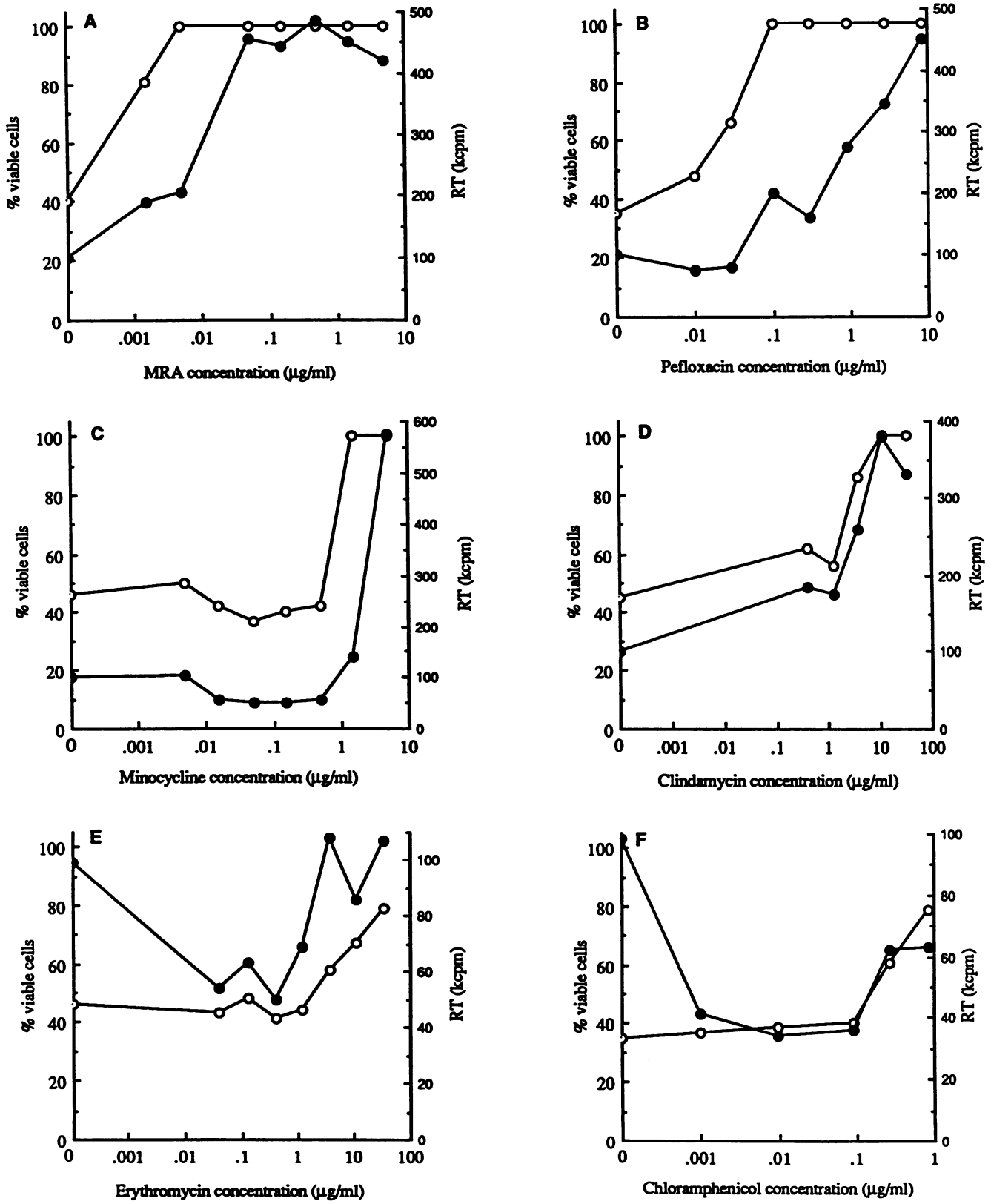

FIG. 1. Activity of antibiotics on CPE and RT activity in CEM cl13 infected with HIV-1. The results were obtained on day 18 after HIV-1 infection. Each value represents the average of three independent points. The antibiotics tested were mycoplasma removal agent (MRA) (A), pefloxacin (B), minocycline (C), clindamycin (D), erythromycin (E), and chloramphenicol (F). The concentration of the antibiotics is given on the x axis. CPE is measured as a percentage of viable cells (left y axis, open circles), and RT activity in the culture supernatant is shown on the right y axis (solid circles).

TABLE 1. Influence of mycoplasma extract on RT activity

Mycoplasma extract	Amt (μl)	RT activity[a] [cpm (% of control)] in indicated vol of HIV-1 suspension:		
		0 μl	1 μl	5 μl
None		680	146,400 (100)	603,014 (100)
M. fermentans	1	499	73,174 (50)	416,179 (69)
	5	475	1,863 (1)	7,714 (1)
M. pirum	1	536	104,967 (72)	189,950 (32)
	5	513	1,527 (1)	4,418 (1)
M. hominis	1	505	185,021 (126)	677,072 (112)
	5	627	151,604 (104)	507,797 (84)

[a] RT activity is expressed as cpm per sample. The value obtained when no mycoplasma extract was added was taken as 100% (control).

TABLE 3. Influence of *M. fermentans* AOU on CPE induced by HIV-1 in U937 cells

Mycoplasma infection[a]	Day of culture after infection with HIV-1							
	6		10		14		17	
	% Viable cells	RT[b] (cpm)	% Viable cells	RT (cpm)	% Viable cells	RT (cpm)	% Viable cells	RT (cpm)
−	98	91,020	95	240,145	100	715,670	100	486,100
+	59	8,940	61	70,200	35	211,000	40	248,000

[a] U937 cells were experimentally infected (+) or not (−) with *M. fermentans* prior to HIV-1 infection.
[b] See Table 2, footnote *b*.

mained negative through 28 days of culture. RT activity and p24 antigen were not detected in any of these cultures, confirming that strain AOU was clearly free of HIV. Then, mycoplasma-free cells and mycoplasma-infected cells were inoculated with mycoplasma-free HIV-1 (100 times the minimum dose needed to infect CEM cl13 cells) or medium alone. The results shown in Table 3 demonstrate that lysis of U937 occurred only when mycoplasmas were present.

M. fermentans can hydrolyze arginine and ferment glucose, and a trivial explanation for lysis of U937 cells would be that mycoplasma growth depleted arginine and/or glucose from the medium (23), resulting in a more pronounced HIV-induced CPE. Therefore, in a control experiment, arginine and glucose were periodically added to the culture medium after HIV-1 infection of both *M. fermentans*-contaminated CEM cells and mycoplasma-contaminated U937 cells. Addition of these nutrients did not alter the CPE (not shown), indicating that cell death cannot be attributed to depletion of arginine and/or glucose.

DISCUSSION

Our results indicate that some species of mycoplasmas, in particular *M. fermentans*, can act synergistically with HIV-1 to promote extensive cell death in some in vitro cell systems, such as CEM cells and U937 cells. However, enhancement of CPE associated with the presence of mycoplasmas in HIV-infected cells cannot be extended as a general rule, because it was not found with H9 cells and normal PBMC.

Antibiotics from the fluoroquinolone and, to a lesser degree, from the tetracycline families were able to inhibit the in vitro cytocidal effect of HIV-1 without affecting viral replication. Although antibiotics greatly reduced single-cell lysis, they did not inhibit lysis of cells involved in syncytium formation. This observation differs from data published by Lo et al., who reported a reduction in syncytium formation when their mycoplasma strain was added to HIV-1-infected lymphocytic A3.01 cells (18). This discrepancy could be due to the fact that the strains of virus and mycoplasma and the cell line differ from the ones that were used in our study. However, these authors (18) have also observed an increased CPE associated with the presence of *M. fermentans* in HIV-infected cells, which is consistent with our results.

Two different species of mycoplasmas, including *M. fermentans*, were isolated from our cell cultures and found to enhance cell killing associated with HIV-1 infection in lymphocytic (CEM cl13) and in promonocytic (U937 and THP1) cell lines.

We also confirmed that the contamination of HIV-infected cell cultures with some mycoplasma species (*M. fermentans* and *M. pirum*, but not *M. hominis*) interferes with RT detection in cell supernatants. One likely hypothesis is that enzymes, such as nucleases, produced by the mycoplasmas are interfering with the RT assay.

Several mechanisms may explain the enhancement of CPE associated with the presence of mycoplasmas. First, mycoplasma contamination could result, for instance, in alteration of receptor expression as a consequence of mycoplasma adhesion (28, 33). This modification could facilitate the attachment of HIV to and/or the entry of HIV into the cells. Second, mycoplasmas have been previously shown to increase the secretion of certain soluble factors, such as cytokines and, in particular, tumor necrosis factor (13), which is known to activate HIV replication (31) and promote

TABLE 2. Influence of mycoplasma infection on HIV-1-induced CPE in different cell lines

Cells	Mycoplasma contamination[a]	Day of culture after infection with HIV-1									
		7		13		15		19		22	
		% Viable cells	RT[b] (cpm)	% Viable cells	RT (cpm)	% Viable cells	RT (cpm)	% Viable cells	RT (cpm)	% Viable cells	RT (cpm)
CEM	−	94	46,557	90	67,411	86	120,069	81	151,312	100	ND[c]
	+	44	19,142	56	38,511	27	82,568	0	ND	ND	ND
U937	−	92	94,042	87	21,686	89	43,763	100	13,333	ND	ND
	+	100	57,508	34	79,102	13	61,280	0	ND	ND	ND
THP1	−	93	1,558	91	13,104	80	43,416	80	27,863	98	29,500
	+	73	278	87	557	75	7,755	42	16,556	40	90,480

[a] The cell lines were shown to be contaminated with mycoplasmas (+) or not contaminated (−) prior to HIV-1 infection.
[b] RT activity in the culture supernatant.
[c] ND, not determined.

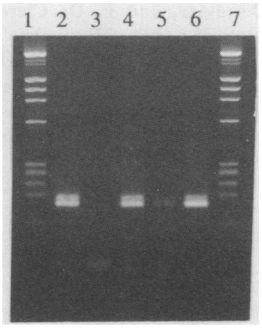

FIG. 2. PCR analysis of mycoplasma strains. The mycoplasmas that were cultivated prior to DNA amplification were strains isolated from contaminated cell lines (CEM cl13, lane 2; U937, lane 3). Other strains of *M. fermentans* were also analyzed: strain AOU (lane 4), PG18^T (lane 5), and K7 (lane 6). The DNA size markers (lanes 1 and 7) are *Hae*III-digested φX174 DNA fragments.

greater CPE in some cell lines (11, 20). Such a mechanism has been suggested in a recent article (6), in which it was reported that a crude extract from the mollicute *Acholeplasma laidlawii* increased HIV-1 replication in chronically infected Molt-4 cells but not in H9 or in U937 cells. However, in our laboratory, the addition of live *M. fermentans* to chronically HIV-1 infected CEM cells did not increase HIV replication, and *M. fermentans* (strain AOU) contamination of different cell lines (CEM and U937) was not concomitant with tumor necrosis factor production (data not shown). Therefore, the tumor necrosis factor hypothesis seems unable to explain by itself the mycoplasmal CPE enhancement. Finally, mycoplasmas could act as cofactors that have been shown to transactivate the HIV-1 long terminal repeat and to increase virus replication in vitro (26). Increased virus production and cytolysis were also associated with mycoplasma contamination of normal mononuclear cells that were infected with cytomegalovirus (37). However, mycoplasma contamination does not necessarily increase virus-induced cytolysis. Pratt et al. (30) reported that poxvirus infection of mycoplasma-infected cells produced a lower virus yield than in non-contaminated cells. But when MRK_{13} (rabbit kidney) cells were infected with the Lister strain of vaccinia virus, mycoplasma contamination induced increased virus replication.

In conclusion, the observations by Lo et al. (17, 18) and the isolation of mycoplasmas from the blood of patients with AIDS in our laboratory (24, 25; this study) indicate that mycoplasmas may be another opportunist in immunocompromised patients and perhaps act as a cofactor in accelerating the development of AIDS. This hypothesis cannot be ruled out and deserves further investigation (8). Moreover, some in vitro biological properties of HIVs and of CD4$^+$ cells (28, 33) can clearly be modified by mycoplasmas. Because these microorganisms are common cell culture contaminants, the effects of mycoplasmas must be considered carefully in HIV studies.

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