Effect of Mycoplasma Infection on Esterase Activity of Human FL Amnion and WI-38 Cells[1,2] (36465)

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Cultured human cells infected with mycoplasma exhibit chromosomal aberrations and dramatic changes in cellular morphology, metabolic rate, and growth characteristics (1). Such extensive cellular alterations may reflect the enzymatic content of the infected cells. In order to investigate the possibility of an association between mycoplasma infection and a change in cellular enzymes, a study was made of the esterase activity of WI-38 and FL cells infected with mycoplasma, comparing them with uninfected cells.

Materials and Methods. WI-38 diploid cell line, derived from female human embryonic lung, and FL human amnion cell line were used throughout all the experiments. The WI-38 cell line was originally obtained from Dr. Leonard Hayflick (Stanford University) and the FL from Microbiological Associates. Both cell lines were propagated on Eagle's basal medium, powdered, (Grand Island Biological Company) containing penicillin (100 units/ml) and supplemented with 10% fetal bovine serum that had been inactivated at 56° for 30 min. Chlortetracycline (50 μg/ml) was substituted for penicillin after several cell passages following the infection with mycoplasma. When the cell layers were confluent, they were passaged in the ratio of 1:2 for WI-38 and 1:3 for the FL. Cells obtained at various passages were harvested and phosphate-buffered saline (pH 7.2) extracts were made following the method described by Bartholomew *et al.* (2). The protein concentration was determined by the biuret method. All cell lines were tested for mycoplasma at the start of the experiment and were found to be free of it. Throughout the experiment the infected and uninfected cells were tested weekly for mycoplasma. The infected cells were consistently positive for mycoplasma, and the uninfected control cells remained negative.

Cultures of mycoplasma were grown in liquid or semisolid medium in test tubes or on solid medium in plastic Petri dishes (Falcon), 60 mm in diameter by 15 mm thick. The basal medium was similar to that of Hayflick (3). It consisted of Heart Infusion Broth (Difco) supplemented with Ionagar No. 2 (Oxoid #L-12) for semisolid (0.1%), or for solid medium (0.9%). Each medium (liquid, semisolid, and solid) contained 15% horse serum (GIBCO), yeast extract (GIBCO, 2%), yeast autolysate (Difco, 1%), potassium penicillin G (Abbott, 100 units/ml), and thallium acetate (0.5%). Specimens tested for mycoplasma are carried for six passages (if negative) in semisolid medium with plating on solid medium for detection of growth. The concentration of mycoplasma in terms of colony-forming units (CFU)/ml was determined from counts on agar plates inoculated with dilutions of 3-day mycoplasma cultures that were used for infecting the cells.

A mycoplasma was isolated from naturally infected FL cells. The infecting microorganism was traced to a lot of mycoplasma-contaminated calf serum. Isolates from the FL cells and from the contaminated calf serum were identified as *Acholeplasma laidlawii* by Dr. M. F. Barile of the Division of Biological Standards, NIH, Bethesda, MD.

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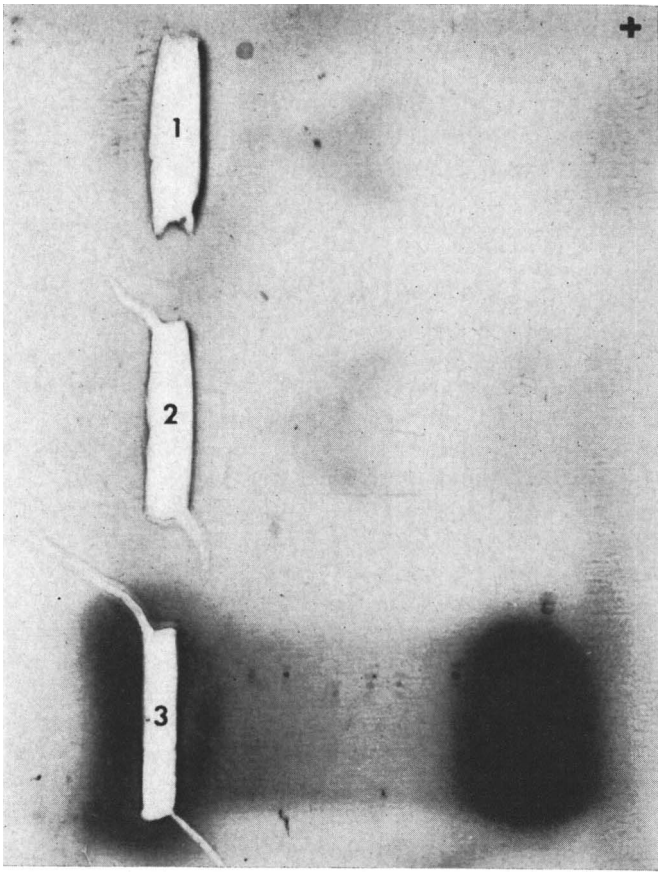

FIG. 1. Agarose electrophoresis of (1) a suspension of *Acholeplasma laidlawii,* (2) an extract of *A. laidlawii,* and (3) FL cell extract at M-2 stained for esterase activity with indoxyl acetate.

This isolate was used in the experiment to infect cells found to be free of mycoplasma infection.

The mycoplasma isolate was grown in liquid medium prior to inoculation of cell cultures. A 3-day culture was diluted in cell culture medium 1:10 and 1:100 and used to infect young, recently subcultured cells. The 1:10 dilution of a mycoplasma culture contained 10^6 CFU/ml and the 1:100 dilution 10^5 CFU/ml. Two or three days after subculturing of tissue cells, the old medium was removed and replenished with new medium containing an appropriate dilution of mycoplasma. The infected cells were incubated for 3–4 days undisturbed. If confluent, they were subcultured, and if not, fresh medium was added. The first passage of the cells after the mycoplasma infection will be referred to in this paper as M-1, the second as M-2, etc. Further propagation of the cells was carried out as described earlier.

Uninfected WI-38 and FL cells were propagated in the same manner as the infected cells and were used as controls. In addition a second control was carried for FL cells. They were "infected" with the same culture of mycoplasma which had been inactivated at 56° for 30 min. No growth of mycoplasma occurred following such treatment.

A study was also performed on the mycoplasma culture for esterase activity. A suspension of mycoplasma in phosphate-buffered saline and an extract of the mycoplasma culture prepared in the same manner as the cell extracts were examined. An agarose electro-

phoresis that was followed by histochemical staining showed very little esterase activity (Fig. 1).

Simple electrophoresis in agarose followed by histochemical staining for esterase according to the method of Uriel (4) was used to compare the esterase enzyme patterns in the cell extracts.

Results. Infection of WI-38 cells with 10^6 mycoplasma CFU/ml proved fatal to the cells. WI-38 cells infected with 10^5 mycoplasma CFU/ml did not show any alteration at first. Four days after the infection the cells were transferred and most of the cultures continued to grow normally although some began to show granularity and reduced growth rate. The cells were tested for mycoplasma and were found positive in the first passage on solid medium. After the third transfer of tissue cells following infection, M-3, all cells exhibited morphological changes. They had become elongated and very granular. The growth rate was greatly reduced; it took 14 days for a monolayer to become confluent.

The extracts of the infected and uninfected WI-38 cells were subjected to agarose electrophoresis and histochemical staining for esterase. Both extracts possessed esterase activity with identical electrophoretic patterns. The infected as well as the uninfected cell extracts had one cathodal and one anodal esterase band with respect to the origin.

FL cells infected with 10^5 or 10^6 mycoplasma CFU/ml did not exhibit any morphological or metabolic changes, but striking changes were observed throughout the experiments in the esterase contents of the cells. They were carried for 16 passages following the mycoplasma infection. After M-6, penicillin was substituted with chlortetracycline in the culture media. Cells were harvested and extracts were made at M-2, M-4, M-6, M-10, and M-16. Mycoplasma was isolated from all of the infected cell extracts, but not from the controls.

Agarose electrophoresis and histochemical staining revealed the presence of several esterase bands in all of the infected cell extracts as well as in the controls. However, the mobilities of the esterase isoenzymes differed greatly in the mycoplasma-infected FL cell extracts. The uninfected control cells possessed a four-band esterase pattern: one cathodal (I) and three anodal (II–IV) bands with respect to the origin. Cells at M-2 exhibited one cathodal (I) and one anodal (II) esterase band, but had lost the more rapidly migrating (*i.e.,* most anodal) bands (III and IV). Furthermore, anodal band II had considerably increased in concentration as compared to the control (Fig. 2). Later mycoplasma-infected cell passages, M-4, M-6, M-10 and M-16, exhibited an esterase pattern rather similar to that of the control cells. They possessed one cathodal (I) and two anodal bands (II and IV), but they were greatly reduced or lacking in anodal band III.

Discussion. This study on the effect of mycoplasma infection of WI-38 indicates that a shift in the esterase isoenzyme pattern was not produced by infection with *A. laidlawii.* However, these findings may not be conclusive, since the cells were propagated for only four passages after infection, and this may not have been sufficient time to observe a change. It was difficult at later passages to make sufficient amounts of extract, because of the drastic reduction in the growth rate following the infection.

A most striking difference between normal FL and FL infected with mycoplasma, was seen with M-2 cell extracts. Two distinct anodal bands (III and IV) that were identified in the uninfected cells could not be demonstrated in the mycoplasma infected cells at M-2. In addition to the loss of the anodal bands, III and IV, the concentration of the remaining anodal band II increased dramatically. This change reverted to a near-normal esterase pattern at M-4 and then remained the same throughout M-16. The cells regained esterase band IV, but were still lacking band III. The intensity of the bands was also very similar to the controls. It appears that the initial effect of mycoplasma infection on cells produced a change in the esterase isoenzyme pattern, but by M-4 the cells had overcome some of this effect of the infection.

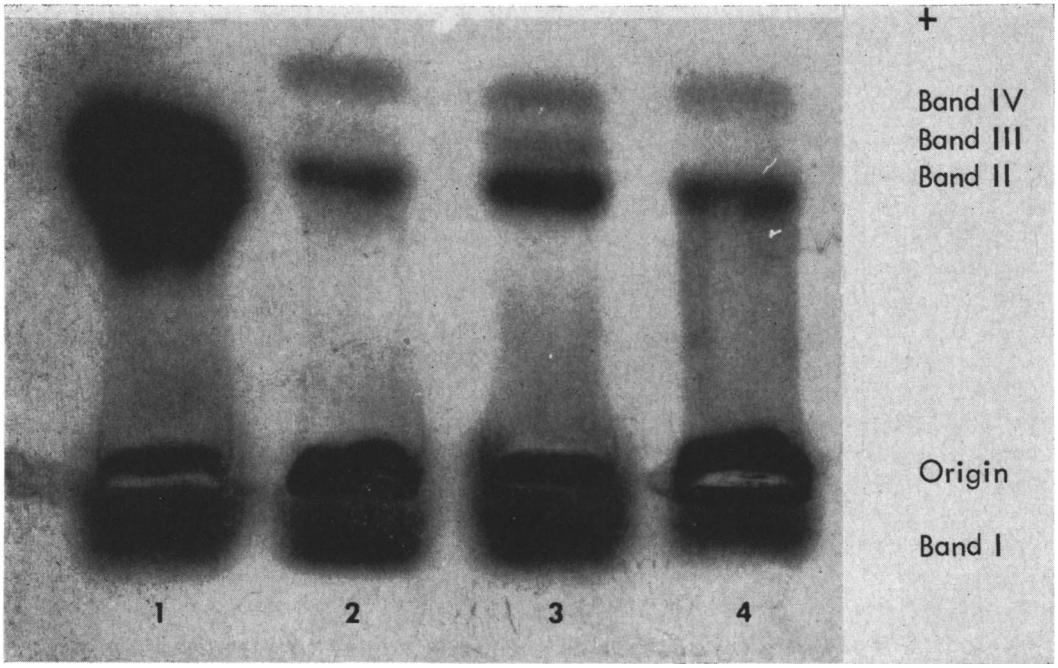

FIG. 2. Agarose electrophoresis of (1) FLM-2 cell extract, 0.340 mg protein, (2) FLM-4 cell extract, 0.348 mg protein, (3) uninfected FL cell extract, 0.350 mg protein, and (4) FLM-6 cell extract, 0.436 mg protein, stained for esterase activity with indoxyl acetate.

Although the alteration in the esterase pattern was reduced, the mycoplasma infection remained. The infection was intracellular, since addition of chlortetracycline (50 μg/ml) to the culture media did not eradicate it. *In vitro* sensitivity tests showed that the mycoplasma was sensitive to the 30-μg disc of chlortetracycline.

The change in the esterase isoenzyme pattern at M-2 was different when the experiment was repeated. On two other occasions there was a marked reduction or absence of band III. There is no doubt that the great increase in band II was genuine in the experiment described. The agarose electrophoresis followed by histochemical staining was repeated a number of times on the M-2 and other extracts varying the position of the M-2 cell extracts on the plate. Both M-2 extracts, the one infected with 10^5 mycoplasma CFU/ml and the second with 10^6 mycoplasma CFU/ml, consistently gave an esterase pattern with band II very concentrated. The development of an altered esterase pattern at M-2 appears to be dependent on cultural conditions that are presently unknown.

The human FL amnion is a permanent cell line and the age of the culture does not seem to alter the enzymatic pattern of the uninfected cells. The changes observed in the esterase isoenzymes of the mycoplasma infected cells appear not to reflect the number of cell passages, but the period of mycoplasma infection on the cells.

Summary. FL amnion cells infected with mycoplasma (*Acholeplasma laidlawii*) showed a marked but transitory change in the pattern of esterase isoenzymes, consisting of a loss of the two most rapidly migrating bands and a great increase in another band. These changes were not observed in mycoplasma infected WI-38.

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