COMPARATIVE ANALYSIS OF MITOTIC ACTIVITY AND CHROMOSOMAL ABERRATIONS IN CELL LINES CONTAMINATED BY MYCOPLASMAS AND IN DECONTAMINATED CELL LINES

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UDC 612.014.3 : [612.6 + 612.6.051]:
576.858.75.081.35 : 576.8.095.383

Mitotic activity and the level of chromosomal aberrations were studied for 3 years in mouse fibroblasts of lines MÉD contaminated with mycoplasmas and in their decontaminated variants. The presence of mycoplasmas, other conditions being the same, reduces mitotic activity but increases the level of chromosomal aberrations.

Recent work has shown that the overwhelming majority of cell lines of normal and tumor origin are infected by microorganisms of the family Mycoplasmataceae [1, 4, 5]. Mycoplasmas are present in most lines as a latent infection with no visible manifestations. However, according to some reports the mycoplasmas contaminating cell cultures can influence the morphology and chromosome composition of the cell, its metabolism, and its physiological properties [6-11].

The question arises whether microorganisms of the Mycoplasmataceae family can affect the mitotic activity and the level of chromosomal aberrations in cell lines contaminated by them. The present investigation was undertaken to study this problem.

EXPERIMENTAL METHOD

Mouse fibroblasts of lines MÉD-14 and MÉD-15 isolated by the authors were used as the test objects [2]. In 1966 mycoplasmas were found in these cell lines, in a concentration of 10^3-10^5 colony-forming units/ml nutrient medium. The mycoplasmas possessed cytopathic properties toward a number of primary cultures [3]. At the beginning of 1967, some of the MÉD-14 and MÉD-15 cultures were decontaminated from mycoplasmas by antibiotics [4]: MÉD-14 by lincomycin and MÉD-15 by terramycin. Since that time the two cell lines have been cultivated in two parallel variants: contaminated and decontaminated. The nutrient medium consistedof Eagle's medium (2/3) and 0.5% lactalbumin hydrolysate solution with 10% bovine serum (1/3). Throughout the period of the experiments the nutrient medium was regularly tested for the presence of mycoplasmas by seeding the culture fluid of both variants of MÉD-14 and MÉD-15 on 0.3% agar made up in a tryptic digest of bovine serum. After decontamination, no mycoplasmas were found at any time in the uninfected variants, whereas seedings of nutrient medium from the contaminated variants always gave growth typical of mycoplasmas in 0.3% agar. The curve of mitotic activity and the number of aberrations in the contaminated and decontaminated variants of the MÉD lines were determined in April, 1967, in February and October, 1968, in April, 1969, and in May, 1970. Cells of both

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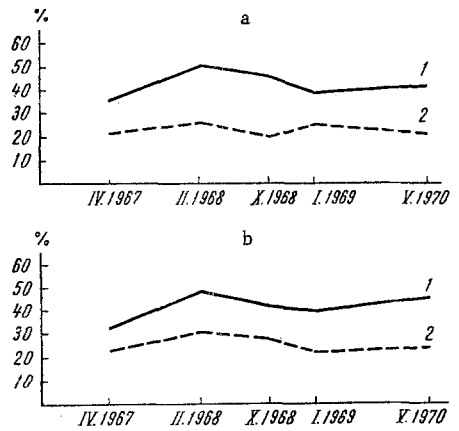

Fig. 1. Level of chromosomal aberrations in MÉD-14 (a) and MÉD-15 (b) cells: 1) contaminated variants; 2) decontaminated variant.

variants were grown in penicillin flasks with halves of cover slips in proportions of 30,000 cells to 1 ml nutrient medium. Every 8 h for 4 days, the two variants were fixed in parallel tests in acetoacetic acid (3 : 1), using 6-8 cover slips at each time. The material was then stained with aceto-orsin. The mitotic index was determined by counting the number of mitoses in 1000 cells. The number of aberrations was determined in the same specimens by analysis of 500 anaphases and early telophases.

EXPERIMENTAL RESULTS

The results of analysis of the mitotic activity in cell lines MÉD-14 and MÉD-15 for 3 years show that the mitotic index of decontaminated variants of the MÉD lines was always 20-25% higher than that of the contaminated variants. This suggests that, despite adaptation of the MÉD lines to the constant presence of the cytopathogenic species of mycoplasmas, constant depression of mitotic activity of the cultivated cell lines took place.

The level of chromosomal aberrations in the two variants of MÉD lines is shown in Fig. 1. In the course of 3 years, the level of chromosomal aberrations observed in the contaminated variants of lines MÉD-14 and MÉD-15 was always higher than in the decontaminated variants. The commonest types of chromosomal aberrations in both the contaminated and decontaminated variants of the MÉD lines were chromatid bridges and paired and single fragments. There was little or no difference between the number of chromatid bridges in the four variants of MÉD lines, but the number of single and paired fragments in the contaminated variants was almost double that in the decontaminated. In addition, the decontaminated lines MÉD-14 and MÉD-15 contained significantly fewer multipolar anaphases with an irregular distribution of chromosomes.

The number of chromosomal bridges observed in the MÉD lines was small, constant, and equal for the contaminated and decontaminated variants.

The level of chromosomal aberrations was also considerable in the decontaminated variants of the MÉD lines. As a result of prolonged cultivation in vitro the MÉD-14 and MÉD-15 lines underwent spontaneous malignant transformation, confirmed biologically by subcutaneous transplantation of the cell suspension into mice of the same line [2]. Tumors grew at the site of transplantation: spindle-cell or mixed sarcomas with invasive growth. The high level of chromosomal aberrations in decontaminated variants of MÉD lines was evidently maintained by virtue of the fact that the tumor cells as such possess latent injuries to their genetic apparatus. These injuries, the number of which depends on the accompanying conditions, are manifested as chromosomal aberrations. However, the presence of mycoplasmas, other conditions being equal, increases the number of chromosomal aberrations and depresses mitotic activity in the cultivated MÉD-lines.

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