LOCATION AND POSSIBLE DEVELOPMENTAL CYCLE OF MYCOPLASMAS AND BACTERIAL L FORMS IN CELL CULTURES

V. V. Neustroeva, Yu. V. Vul'fovich, and G. Ya. Kagan

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With the use of the vital dye Chlorazol Black E the location and behavior of five species of mycoplasmas and a stable L form of group A β-hemolytic streptococcus were studied in various continuous cell lines. The mycoplasmas and L forms were shown to pass through a definite developmental cycle in the cells; initially they were located extracellularly and on the cell surface, later intracellularly, when they multiplied intensively; later still they were again found on the cell surface and extracellularly. This cycle was shown to depend on the type of infection. The character of localization was shown to depend both on the species of both agent and culture.

KEY WORDS: mycoplasma; L forms of streptococcus; cell culture; developmental cycle.

Cytochemical [2, 6] and immunochemical [1, 5, 7] methods of detecting mycoplasmas and bacterial L forms in use at the present time, although capable of showing the location and the microstructural features of the antigenic components of the mycoplasmas and L forms, do not shed light on their viability. Vital staining of the microorganisms is evidently the most promising method from this standpoint.

Previous investigations showed that the dye Chlorazol Black E can be used for intravital staining of mycoplasmas and bacterial L forms and their subsequent detection in cell cultures [4].

The location and possible developmental cycle of mycoplasmas and bacterial L forms in cell cultures were studied in different types of infection caused by them.

EXPERIMENTAL METHOD

Experiments were carried out on continuous lines of pig embryonic kidney (SPEV), human kidney (RH), and green monkey kidney (MA-104) cells and on mouse fibroblasts [1]. All cultures were free from contamination by mycoplasmas. The cells were grown on coverslips in the usual way on medium on 199 with 10% bovine serum. A 24-to 48-h culture was used for infection.

Standard strains of mycoplasmas were used: M. gallisepticum S_6, M. laidlawii, M. laidlawii var. inocuuni, M. sp. 200, isolated from hens and identified as M. gallinarum, M. sp. 110, isolated from mice and identified as M. arthritidis, and also the L form of β-hemolytic streptococcus, strain L-406, isolated from a patient with rheumatic carditis [3], and maintained by prolonged passage in the laboratory.

The mycoplasmas were grown in broth or on 0.3% agar, the basis of which was a tryptic digest of bovine heart muscle with the addition of 10% yeast extract and 10-15% bovine or horse serum. The L form also was grown on broth or agar, but with the addition of horse serum and NaCl as osmotic stabilizer. A 3-to 4-day culture was used for infection.

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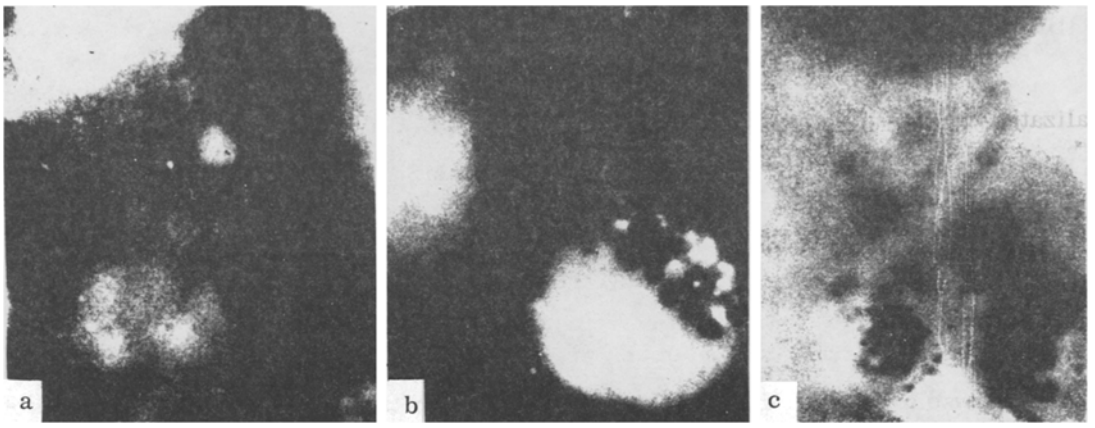

Fig. 1. Cell cultures infected with mycoplasmas: a) SPEV infected with M. gallisepticum S_6; 24 h after infection; b) SPEV infected with M. gallisepticum S_6, 3rd day after infection; c) MA-104 infected with M. laidlawii, 10th day after infection. Stained with acridine orange, objective 90 ×, ocular homal 3 ×.

Chlorazol Black E in a concentration of 1:1000 was mixed in the ratio of 1:1 with the culture of mycoplasmas and L forms, left for 1 h at room temperature, and the cell culture was then infected with 0.2 ml of the mixture. The infecting dose was 10^3-10^4 colony-forming units (CFU)/ml. A cell culture inoculated with a mixture of Chlorazol Black E and nutrient medium was used as the control. The cells were incubated at 37°C and examined daily in the luminescence microscope, without fixation, after preliminary staining with acridine orange (1:100,000).

EXPERIMENTAL RESULTS

Mycoplasmas which differed in the character of their behavior in cell cultures were used in the experiments. For instance, M. gallisepticum S_6 induced an acute infection in all cultures with well-marked destructive changes, whereas the other mycoplasmas had no cytopathic action and induced a latent infection. The L form of streptococcus produced slight cytopathic changes in the SPEV culture and caused the appearance of giant multinuclear cells in the MA-104 culture; in the other cultures the infection was latent in character and was unaccompanied by any visible morphological changes.

On the first day after infection the mycoplasmas and L forms, irrespective of the type of infection produced by them or the type of cell culture, were located outside the cells or on their surface as homogeneous conglomerates of different sizes, and only very rarely were they found as single black granules in the cytoplasm (Fig. 1a). On the second to third day the number of conglomerates was reduced, and later they disappeared completely, when small granules, diffusely scattered in the cytoplasm, became more numerous (Fig. 1b). Meanwhile dark gray oval formations appeared at infrequent intervals in the enlarged cells. These formations existed for 2-4 days depending on the type of infection (less in acute and more in latent forms), after which they disappeared and the number of small granules in the cytoplasm continued to increase. Initially they were located beneath the cytoplasmic membrane, often they were perinuclear in their location, but later (5th-7th days) they frequently filled the whole cytoplasm. On the sixth to ninth day, depending on the type of infection (earlier in acute, later in latent), besides small granules, conglomerates of different sizes consisting of small granules appeared in the cytoplasm together with gray oval formations similar to those observed on the second to fourth day; the latter were found more frequently in latent infection. In the later stages (9-15th days) the number of conglomerates increased and most of them were found on the surface of the cells or outside them (Fig. 1c); this process again was more marked in latent than in acute infection. In acute infection small granules predominated in the cytoplasm, and later (sixth to eighth days) extracellularly also.

These results may indicate that mycoplasmas and L forms of bacteria pass through a definite developmental cycle in cell cultures. Initially (first to second day) their localization is extracellular or on the surface of the cells, evidently on account of adsorption of microorganisms on the cells. Later (second to sixth day) the infectious agent probably penetrates into the cells and multiplies there, as shown by a marked increase in the number of microorganisms observed in the cytoplasm. Later still (eighth to tenth day) they

leave the cells, and the mycoplasmas and L forms are again found on the surface of the cells or outside them. The character of their localization depended on the type of cell culture and of infectious agent. For instance, M. laidlawii in SPEV, RH, and L cells were indistinguishable in the character of their intracellular localization from other species of mycoplasmas (occurring beneath the membrane, in the cytoplasm, and in the perinuclear region), and in the MA-104 culture they were also found in the nucleus. The cycle described above was shown to depend on the type of infection, being more marked and following a longer course in the latent type of infection.

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