BEHAVIOR OF MYCOPLASMAS ISOLATED FROM BIRDS IN VARIOUS CELL CULTURES

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The behavior of six strains of mycoplasmas isolated from birds were studied in primary cultures of chick embryonic fibroblasts, chick embryonic kidney, quail embryonic fibroblasts, and transplantable lines of Spev, Hep-2, and RH cells. Mycoplasma gallisepticum S_6 possessed the strongest cytopathic action, inducing a cytopathic effect in all cultures. Three strains (M. iners pg-30, M. sp. 198, and M. sp. Br-7) induced changes only in the primary cultures, while two strains (M. gallinarum Tu and M. sp. 190) produced no visible morphological changes.

Mycoplasmas, including those of the avian species, are very frequent contaminants of cell cultures, in which they can induce the most varied changes – from a latent infection and slight morphological changes to transformation, degeneration, or complete lysis of the cells [1, 5]. Information on the behavior of mycoplasmas discovered in birds in cell cultures is extremely scanty, but what there is shows that these strains can multiply in cell cultures and induce cytopathic changes [2-4].

The object of the present investigation was to study the behavior of some strains of mycoplasmas isolated from birds in primary trypsinized cultures and in transplantable cell lines.

EXPERIMENTAL METHOD

Reference strains of mycoplasmas Mycoplasma gallisepticum S_6, Mycoplasma gallinarum Tu, and Mycoplasma iners, and serologically unidentified strains of mycoplasmas Mycoplasma sp. 190, M. sp. 198, and M. sp. Br-7, isolated from birds obtained from different areas of the country where mycoplasmosis is prevalent, were used in the investigation.

The mycoplasmas were cultivated on semiliquid (0.3%) agar, based on a tryptic digest of bovine heart muscle with the addition of 10% yeast extract, 10% bovine serum, and 100 units/ml penicillin.

The investigations were carried out on primary cultures and transplantable cell lines. The primary cultures included chick embryonic fibroblasts, chick embryonic kidney, and quail embryonic fibroblasts. The transplantable cell lines were Hep-2, Spev, and RH. All were first freed from contamination with mycoplasmas, and periodic control tests confirmed their absence. The cells were grown by the usual method, the primary cultures in a 0.5% solution of lactalbumin hydrolyzate with 5% bovine serum, and the transplantable lines in medium No. 199 with 10% of the same serum. The cells were infected as a 2-3-day culture with a fully formed monolayer. For the morphological tests the cells were seeded into tubes with slides, which were fixed daily after infection in Carnoy's mixture and stained with hematoxylin and eosin.

A 3- or 4-day culture of mycoplasmas was used to infect the cells, and was added in a dose of 0.1 ml to the tubes. The tubes were then incubated at 37°C for 10 days.

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EXPERIMENTAL RESULTS

Depending on the character of their behavior in the cell cultures, all the strains of mycoplasmas which were studied could be divided into two groups: 1) mycoplasmas inducing cytopathic changes (M. gallisepticum S_6, M. iners, M. sp. 198, and M. sp. Br-7) and 2) mycoplasmas not inducing cytopathic changes (M. gallinarum Tu and M. sp. 190).

The cells with cytopathic action differed among each other in the time of onset and the intensity of the cytopathic changes.

The most marked cytopathic action was shown by M. gallisepticum S_6, which induced cytopathic changes in all the cultures studied. The sharpest changes were observed in the primary cultures, in which total destruction of the monolayer was observed 2-3 days after infection, accompanied by a marked shift of the pH of the medium to the acid side. In the transplantable cell lines the cytopathic changes began to develop later, on the 3rd-4th day after infection, and degeneration was incomplete. As in the primary cultures, the medium became strongly acid.

The remaining three strains (M. iners, M. sp. 198, and M. sp. Br-7) induced cytopathic changes only in the primary cultures, and these began considerably later than after infection with M. gallisepticum S_6 (on the 3rd-6th day), and the degeneration produced by M. iners and M. sp. Br-7 as a rule was incomplete. The cytopathic action was not accompanied by acidification of the medium.

Whereas the strains studied differed from each other in the time of onset and the intensity of the cytopathic changes which they induced, in the character of their cytopathic action they were indistinguishable. In primary cultures vacuolation, loosening of the layer, and subsequent destruction were observed, while in the transplantable lines the degeneration began at the periphery of the layer and was only partial, for 30-50% of the cells remained attached to the glass.

The observation showed that the mycoplasmas were not merely preserved, but also that they multiplied in the cell cultures tested. This was shown by their ability to induce a cytopathic effect over a long period of time (5-10 passages for various cultures) and by the positive results of seeding from infected cultures throughout this period.

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