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Редотова, Т. М.  
Сревителна култура на туберкулоза  
(Cooperatively) and vaccine strains of Pasteurella  
(Francisella) in an embryo tissue culture  
Zhurnal Mikrobiologii i Immunobiologii 1963(12):84-88.  
443,3 24  
(In Russian)

In the microbiologists' arsenal of experimental methods the appearance of in vitro tissue (cell) cultures has made possible the study of the interrelationships of pathogenic bacteria and isolated cells of the macroorganism. At the present time study of tissue cultures in connection with these particular problems of the pathology of infectious diseases (Begin p.85) is primarily concerned with the character of intracellular behavior of pathogenic bacteria. Thus, it has been established for several microbial species that after penetration of the cell virulent bacteria multiply in the cytoplasm at an intense rate, and, in contrast, the avirulent bacteria are not only incapable of multiplying, but cannot even exist for long outside of the cell. The ability of intracellular multiplication in relation to virulence was shown for all three types of Brucella in tissue culture cells of chick embryos (Holland and Pickett, 1956), for Staphylococcus in human leukocytes (Hogers and Torpsett, 1952), and for Bacillus typhimurium in chanted cells of tissue cultures of monkey kidneys and in macrophages of mice (Furness, 1958), etc.

The study of the interrelationships of tularemia pathogens and cells in vitro up to the present has also been concerned chiefly with the character of intracellular bacteria. In 1958-1959 Shepard observed in HeLa cells a direct ratio between the rate of intracellular multiplication and the virulence of these bacteria. These data were later confirmed by Merriott and co-authors (1961) in experiments with cultures of mouse fibroblasts of cellular strain L. They studied a number of strains of Pasteurella tularensis of varying virulence, including with the highly-virulent 525, and concluding with the avirulent strain 538. According to their data, the rate of intracellular multiplication increased in proportion to an increase in the virulence of the tularemia bacteria. The lower the virulence of the strains, the less their multiplied within the cells. Bacteria of strain 538 which had lost their virulence did not multiply at all in cells and, being in the cytoplasm, quickly perished.

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The direct relationship between the intracellular multiplication rate of tularemia pathogens and their virulence was established by Stefanye and co-authors (1961) in leukocytes of peritoneal exudate of guinea pigs.

The authors did not observe marked morphological variations in the cytoplasm of cells with tularemia bacteria of various degrees of virulence with their multiplication. In studying the highly-virulent strain 525 in a culture of mouse fibroblasts of cellular strain L, Merriott and co-authors noted the appearance in the culture of accumulations of cells whose cytoplasm was filled with tularemia bacteria. Later they observed the appearance within these accumulations of a large number of extracellular bacteria, as if they had been sorted on the surface of the cells.

The present work is devoted to the study of the interrelationships between tularemia bacteria and cells of tissue cultures as a whole. We studied the character of the cytopathic activity of tularemia bacteria and the reverse action of tissue culture cells on the bacteria.

The work was conducted on a single-layer human embryo tissue culture. In a preceding work on the comparative study of the interrelationships of anthrax pathogens of varying virulence and human embryo tissue culture cells, it was explained that in growing cell cultures with the use of antibiotics, a certain amount of the antibiotics remained even after many washings and was capable of affecting the bacteria introduced into these cultures. Therefore, beginning with the initial stage of taking the embryonic material at the hospital, we did not use antibiotics in any stage of preparing the tissue cultures.

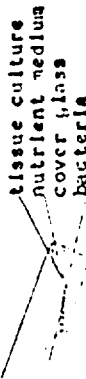
The tissue cultures were grown on cover-glass slides in test tubes. In order to be certain of the definite location of the slide with the culture in the test tube, throughout the experiment, markings on the glass were used and the slide was thus oriented from the time of its insertion. During the experiment the slide cultures were removed from the test tubes, the tissue was fixed in ethyl and stained with the Romanov-Giemsa stain. Cell cultures were grown and inoculated in a medium of lactalbumin hydrolysate, to which a 0.10N solution of calf serum was added; this was inactivated at a temperature of 56-58°C for 30 minutes. Two strains of Pasteurella tularensis were used in the work: the standard virulent strain (no. 525), isolated from ticks by Olsufrey in 1949, and vaccine strain 154, obtained by Galickii in 1941 and reactivated by Finelyanova in 1954 by passage through guinea pigs and mice. With the inoculation of strain 503 subcutaneously, the dosage (Dalm) for mice and guinea pigs was 1 microbial cell, for white mice--100 million bacteria, and for rabbits--approximately 10 billion (by visual standards). Dalm of the vaccine strain in the subcutaneous inoculation of mice was 1 billion; with the injection

of doses of 100 to 1 million bacteria subcutaneously, thirty to fifty percent of the mice died.

A 24 hour tularemia culture grown on glucose-cystins-blond agar was used for inoculating cell cultures. Bacterial suspensions were prepared for in the medium for growing tissue cultures. Bacterial concentration was established according to the visual standards of the State Control Institute with suitable re-calculation (10 turbidity units of the standard corresponded to 5 billion microbial cells per ml. of vaccine strain suspension and 10 billion per ml. of the virulent strain). Cell cultures were inoculated with a dose of 300 million bacterial cells.

The interrelationships of tularemia bacteria with tissue culture cells were studied in two ways: first, of all, the character of the immediate (direct-on contact) cytopathic activity of the bacteria on human embryo tissue culture cells and that of the tissue culture cells on the bacteria; secondly, the action (toxic-through a medium) of products and metabolites of Past. tularemia on the cells was studied. In each case modified versions of the methods of observation and research were used. In this article data is given on the study of the direct cytopathic action of Past. tularemia on cells and of the cells on the bacteria,

I - 24-hour exposure.



II - subsequent culture.

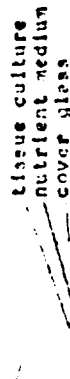


Figure 1] Diagram of the experiment for studying the interrelationships of bacteria and cells in human embryo tissue culture.

The human embryo tissue culture, located on the upward layer and inoculated with the suspension of tularemia bacteria, was kept for 24 hours at 37°; the culture was then washed three times and fresh nutrient medium was added. The washing was done in order to remove from the culture as completely as possible extracellular bacteria which could later complicate observations of intracellular bacteria. In order to eliminate subsequent settling of bacteria which were multiplying in the nutrient medium on the tissue culture cells, after the 24-hour exposure the slides were turned upside down and with the tissue layer downward it was kept in an incubator until the conclusion of the experiment (figure 1). The nutrient medium in the test tubes was replaced daily. Since the tularemia bacteria multiplied slowly in the nutrient medium which we used, daily replacement of the medium was sufficient for its practically continuous duration for the entire experiment of culturing the cells. (Bagin p.67)

Cover glasses were taken out of part of the test tubes daily, were washed in physiologic salt solution in order to remove bacteria which were insecurely adhering to the cell culture, and were fixed and stained as described above. Observations were carried out for 10-12 days.

Forty-eight hours after inoculation, it was possible to observe in microscopic examination of the preparation, various stages of intracellular bacterial colonization of virulent and vaccinal strains. At first they were arranged in chains in the cell cytoplasm (figure 2); by 72-96 hours they had completely filled up the entire cytoplasm, growing around (by-passing) the cytoplasmic vacuoles and leaving of free (figure 3). In the process of intracellular multiplication of vaccinal strain bacteria, cell nuclei retained their normal form, and for the virulent bacteria the cell nuclei as a rule were destroyed (figure 4). Destruction of the nuclei began with the partial colonization of cells with the virulent bacteria.

The intensive rate of multiplication of bacteria in the cells caused changes of form in the cells; they took on an irregular, oval form and appeared as sacks stuffed with bacteria (figure 5).

As a rule the cells filled with bacteria were destroyed. With intracellular multiplication of virulent bacteria the first sign of injury to the cell was destruction of the nucleus; this made further existence of the cell impossible. Vaccinal bacteria which filled the cell cytoplasm had no marked effect on the morphology of the nucleus and in this case cells overflowing with bacteria evidently ruptured mechanically, although it is impossible to exclude the fact that such a cell can be destroyed as a result of the disturbance of normal course of its cytoplasmic processes. It is necessary to mention that with intracellular bacterial multiplication of the vaccinal strain, the process does not always end with complete colonization of the cell.

In a number of cases we observed swelling and lysis of bacteria within the cells.

Foci of multiplying tularemia bacteria later arose in the tissue culture. In considering the conditions of the experiment and, having excluded (for all practical purposes) the settling of non-motile bacteria on the cells after 24 hours of contact, we assumed that these foci arose in places where cells filled with bacteria were destroyed. In experiments with virulent bacteria the foci had arisen by the 5th-6th day after inoculation, and in experiments with the vaccinal strains--not until the 8th-9th day. As a rule the manifestation of foci in experiments with virulent bacteria was preceded by the accumulation of cells filled with bacteria. Evidently the colonization of adjacent, re-infected cells began with the originally colonized and destroyed cells, and the newly-infected cells were gradually destroyed, while infecting the neighboring cells, etc. Thus arose the focus of infection of the tissue culture with virulent Past. tularemia (figure 6). The bacteria in the focus multiplied at an increasing rate on the detritus of the destroyed cells, within, and on the surface of the cells. The center of this focus became necrotized in proportion to the amount of growth. (figure 7).

In the focus induced by the vaccinal bacteria strain, the rate of injury to the tissue was of a lesser degree.

It must be noted that bacteria in the foci were securely fixed on the surface of the cells.

Part of the bacterial cells fixed on the cell surface swelled up and underwent lysis. Apparently this was caused by the presence of non-specific bacteriolytic factors released by the cells. (Begin p.88)

CONCLUSIONS

1. Both strains of Pasteurella tularensis penetrate into the cells of the human embryo-tissue culture and multiply in their cytoplasm.
2. Destruction of the cell nuclei was observed with the intracellular multiplication of bacteria of the virulent strains; cell nuclei retained their normal form upon multiplication of bacteria of the vaccinal strain.
3. Foci of multiplying tularemia bacteria arose at the site of cells in the tissue culture which were destroyed due to intracellular bacterial multiplication. Tissue in the center of the foci was necrotized in proportion to the rate of growth. Foci induced by vaccinal strains of the bacteria were manifested in the tissue culture later than those induced by virulent strains, and were characterized by less destruction of tissue.

4. Along with the above, the secure fixing of focal bacteria to the surface of cells and the lysis of part of the fixed bacteria, regardless of their virulence, were observed.

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