DISTANT INTERCELLULAR ELECTROMAGNETIC INTERACTION BETWEEN TWO TISSUE CULTURES

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UDC 612.014.426-085.23

Experimental data on distant intercellular electromagnetic interaction between two tissue cultures when one of them is exposed to factors of biological (viruses) or chemical (mercuric chloride) nature are presented; the characteristic response of the "intact" culture is in the form of a "mirror" cytopathic effect.

KEY WORDS: distant intercellular electromagnetic interaction; "mirror" cytopathic effect.

The existence of very weak intrinsic emission of radiation from biological objects (biochemiluminescence) is now generally accepted [1-4]. So far there have been few investigations aimed at determining the possible role of electromagnetic radiation in biological systems, although the possibility that biological objects can emit intrinsic radiation of different ranges has been demonstrated [5-7]. There is reason to suppose that electromagnetic interaction is a general principle of interchange of information among biological systems. Quanta with different frequency characteristics may perhaps be carriers of information.

Since 1966 the authors have studied the phenomenon of distant intercellular interaction due to electromagnetic radiation in the UV band [8-11]. The method of biological detection suggested by A. G. Gurvich has been used in our investigations to study the biological action of electromagnetic radiation in the biosystem.

Since we were interested to discover whether the electromagnetic radiation of cells performs a signal function, it was necessary to choose a state of the cells which could be clearly analyzed with the aid of the biological detector. A suitable object from this standpoint was a tissue culture infected with different viruses (Coxsackie A-13, the classical fowl pest virus — FPV) or treated with mercuric chloride. In these cases the specific action of the viruses and mercuric chloride could be analyzed on the basis of their cytopathic action and immunologic changes. The experiments were planned so that the tissue culture infected with viruses or injured with mercuric chloride was the source of a specific signal, encoded in very weak radiation of the cells, and the intact tissue culture (not infected with virus) would serve as detector of this radiation. In the cells of the intact culture (henceforward designated the "mirror" tissue culture), in optical contact with the affected tissue culture, all morphological features of the extremal states specifically characteristic of the corresponding agent, developed. These morphological features are henceforward described as the "mirror" cytopathic effect (CPE).

EXPERIMENTAL METHOD

The tissue culture serving as the testobject was grown in special chambers on quartz or glass slide supports of different thickness (from 0.2 to 2 mm), soldered to a ground-glass joint. The transmitting capacity of the quartz slides in the region of 280-320 nm was 70-90%. The maximum of transmitting capacity of the glass slides lay in the visible region, starting from 440 nm. Primary cultures of human and chick embryonic fibroblasts and also transplantable monkey kidney tissue cultures were used.

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0007-4888/80/8903-0345$07.50 © 1980 Plenum Publishing Corporation

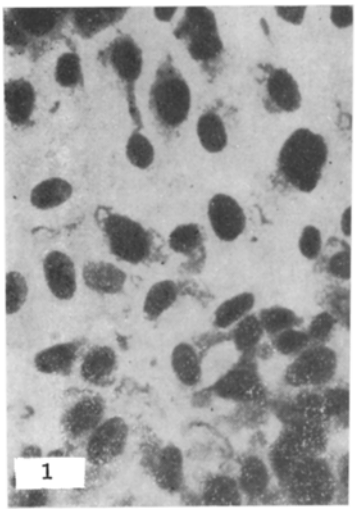
After a monolayer had formed on the floor of the chambers, the chambers with the introduced harmful factor were mounted in pairs with the intact slide supports and fixed to a revolving drum perpendicularly to the axis. The drum was placed inside a darkened thermostat (37°C) and rotated together with the chambers at a speed of 25 rpm. The cells in the two chambers were thus bathed equally with nutrient medium, did not dry, and were adequately nourished. All experiments were accompanied by a control for detection of spontaneous degeneration of the tissue culture cells. After 2-4 days the chambers were removed and dismantled, the slide supports with cells growing on them were sealed off, and after fixation and staining, the cultures were examined morphologically. The CPE was calculated from the ratio of the number of dying cells to the total number of cells and from the type of morphological changes. The CPE was taken to be weakly positive if the ratio was 1:10, average if it was 1:5, and strongly positive at 1:2. Altogether about 1500 experiments, together with controls, were carried out.

EXPERIMENTAL RESULTS

1. The "Mirror" CPE after Infection with Coxsackie A-13 Virus (350 Experiments). The cytopathic action of Coxsackie A-13 virus consists of breaking up of the monolayer and the appearance of round cells. Later the round basophilic cells undergo pycnosis: They shrink, become polygonal, and their nucleus becomes strongly hyperchromic. The pycnotic cells then disintegrate, and pycnotic "fragments" can be observed along with solitary intact cells. In the "mirror" cell cultures, inhibition of mitosis could be seen. The monolayer also was broken up with the appearance of pycnotic hyperchromic cells. Cells of this type later disintegrated, and in the "mirror" cell cultures, inhibition of mitosis could be seen. Cells of this type later disintegrated, and in the "mirror" cultures essentially the same evolutive forms of degeneration were observed as in cultures infected with virus. The tempo of development of degeneration in the "mirror" chambers was about 12-14 h behind. All experiments were accompanied by an appropriate control for detection of spontaneous degeneration in the uninfected culture. In chambers in which simple glass was used as the slide support, no "mirror" CPE developed. During passages from the infected chambers, A-13 virus was regularly isolated. Even after repeated passages, no virus could be isolated from the "mirror" chambers, whether with a positive or with a negative "mirror" CPE. A positive "mirror" CPE occurred in 74% of cases (Figs. 1-3).

2. "Mirror" CPE after Infection with FPV Virus (453 Experiments). The experiment with FPV followed the same scheme as that with Coxsackie A-13 virus. In all the infected chambers degeneration characteristic of classical FPV was observed. It took the form of breaking up of the monolayer, rounding of many cells, and a tendency for the cells to form clusters. At the same time large, symplasmatic structures were formed, in the peripheral zone of which the nuclei formed a palisade. Later individual cells became shrunken. In uninfected "mirror" chambers in optical contact with the infected chambers, the presence of a "mirror" CPE was observed in 78% of cases. In the "mirror" culture many single rounded basophilic cells appeared, many of which later did not lose contact with each other, so that the monolayer became divided into a series of large cell complexes resembling bunches of grapes. The cytoplasm of the cells in these complexes was highly vacuolated and their nucleus was condensed. Passage of the culture fluid from infected and noninfected chambers showed virus to be present only in the infected chambers. The hemagglutination test with fowl erythrocytes and culture fluid from chambers infected with FPV revealed hemagglutinins in the infected chambers in titers of 1:40, 1:80, and 1:160. Culture fluid from the uninfected chambers, even if a "mirror" CPE was present, did not give a positive hemagglutination test, evidence of absence of the virus.

3. "Mirror" CPE after Poisoning Cells with Mercuric Chloride (412 Experiments). To study the universality of the type of intercellular connections thus revealed, toxic injury to cells of tissue cultures of chick and human fibroblasts with mercuric chloride was chosen as a different model of cytopathic states. A toxic dose of $HgCl_2$ was used, causing death of the tissue culture cells after 2-3 days through blockade of respiratory enzymes. In the chambers with $HgCl_2$ a cytopathic effect developed with disintegration of the monolayer and granular and vacuolar degeneration of the cells and karyopycnosis. The process ended with total death of the monolayer. The "mirror" CPE also consisted of disintegration of the monolayer, vacuolation of the cytoplasm, and karyopycnosis (78% of positive experiments). The achievement of a "mirror" CPE in the experiments with $HgCl_2$ required the experiment to be performed under the same conditions as when viruses were used.



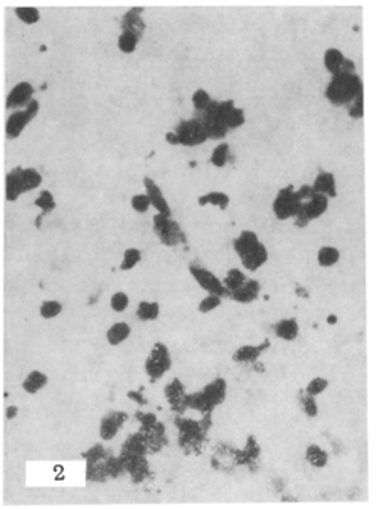


Fig. 1 Fig. 2

Fig. 1. Normal culture of human embryonic fibroblasts. Here and in Figs. 2 and 3: hematoxylin-eosin; 400×.

Fig. 2. Coxsackie A-13 virus. Total death of cell culture. Trypsinized cells.

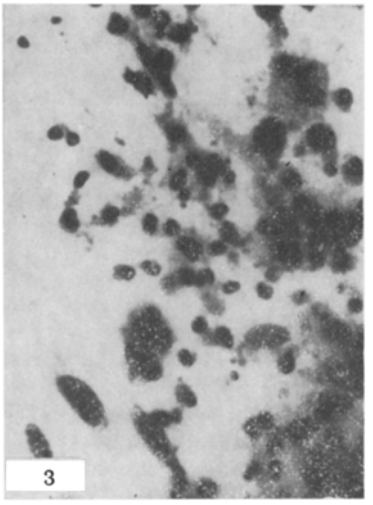


Fig. 3. "Mirror" cell culture. Large area of monolayer. Nuclei pycnotic. Many cells disintegrated, some of them rounded and hyperchromic.

A control was set up for detection of spontaneous degeneration. In the chambers in which simple glass was used as the slide support, no "mirror" CPE of $HgCl_2$ developed. Statistical analysis of the results pursued two purposes: 1) determination of the probability of obtaining a "mirror" CPE for 95% confidence limits (5% level of significance of Pearson's criterion), 2) determination of similarity or difference between the action of viruses and $HgCl_2$ by comparison of alternative distributions using Pearson's criterion.

The results showed that a "mirror" CPE can be reliably found within confidence limits of 90 to 65% and at a 95% level of significance. The effectiveness of action of the two viruses and of $HgCl_2$ did not differ significantly as regards the production of a "mirror" CPE.

The experiments thus showed that in the presence of optimal contact between two isolated

tissue cultures distant interaction takes place and is expressed as the repetition of the morphological features of the cytopathological process induced in one of the cultures by means of viruses or mercuric chloride, in the other intact tissue culture, or in other words, a "mirror" CPE takes place.

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ROLE OF GENETIC DIFFERENCES BETWEEN MOTHER AND FETUSES IN THE DEVELOPMENT OF A GRAFT VERSUS HOST REACTION INDUCED IN THE MOTHER

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UDC 616-056.43-02:612.02.017

Development of the graft versus host reaction (GVHR) was studied in female (CBA×C57BL/6)F_1 mice during pregnancy, and after birth or the day before mating with syngeneic, semisyngeneic, and allogeneic males. The development and outcome of the GVHR in the female mice was shown to depend on genetic differences between the donors of transplanted lymphocytes and the fetuses and also on the time of induction of the GVHR. If lymphocytes from C57BL/6 mice were injected into (CBA×C57BL/6)F_1 females after parturition or on the day before mating with males of the parental CBA line, pregnancy led to enhancement of the GVHR; if lymphocytes were injected during pregnancy, an increase in resistance to the BVHR was observed. In the case of mating with males of the contralateral parental line C57BL/6 (syngeneic with respect to the lymphocyte donors) pregnancy did not affect the development of the GVHR regardless of the time when the cells were injected.

KEY WORDS: immunopathology of pregnancy; mother–fetus; graft versus host reaction; genetic differences.

During pregnancy the maternal immune system is exposed to the action of different cells and subcellular factors carrying genetic information of the fetus. The biological signifi-

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 3, pp. 340-342, March, 1980. Original article submitted June 6, 1979.

0007-4888/80/8903-0348$07.50

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