CYTOLOGICAL STUDY OF TRANSPLANTABLE LINE OF PIG EMBRYONIC KIDNEY CELLS PERSISTENTLY INFECTED WITH TOXOPLASMAS

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Infection of an RES culture by toxoplasmas does not seriously disturb the vital activity of the cells and does not cause them to degenerate rapidly. Changes observed in the morphology of the host cells are evidently mainly the result of purely mechanical pressure of the vacuoles on the nucleus and cytoplasm, although extreme forms of disturbance of cell morphology are possibly associated with more severe injury. Complete "self-sterilization" of the cultures evidently does not take place for, despite a sharp decrease in the number of parasites, the possibility of reinfection of the culture by toxoplasmas in the later stages after initial infection cannot be ruled out.

KEY WORDS: cell culture; toxoplasmas; persistence of infection.

Multiplication of toxoplasmas and their action on the host cell have been investigated previously in many types of cell cultures [1, 7, 9]. However, the problems of the choice and study of a model cell culture in which chronic or latent infection can take place, a typical feature of toxoplasmosis, remain tasks of some urgency.

Cell cultures adequately resistant even to virulent strains of toxoplasmas and giving rise to prolonged persistence of infection are of the greatest interest. There are isolated data on the development of the parasites under these conditions [2, 7], but virtually no information on the cytological characteristics of the host cells.

A culture of RES cells, a transplantable line of pig embryonic kidney tissue [2, 3], was chosen as the test object. The characteristic feature of this line of cells is its high adhesive power combined with comparatively low mitotic and metabolic activity and, in particular, its diploid constitution [3, 8]. These properties of the RES strain were the reasons for choosing it in order to study toxoplasma infection and its effect on the karyotype of the host cells [2].

This paper describes a cytological analysis of the response of the host cell to infection.

EXPERIMENTAL METHOD

The object for infection with toxoplasmas (Toxoplasma gondii, strain RH) consisted of 5-6-day RES cultures grown on coverslips in penicillin flasks and infected with toxoplasmas by methods described by the writers previously [2]. The preparations were fixed in Nikiforov's mixture every 24 h after infection for 14-19 days. The Romanovsky–Giemsa staining method was used. Mitotic activity was determined in the usual way [6] in 2000 cells in different parts of the specimen and expressed per 1000 cells.

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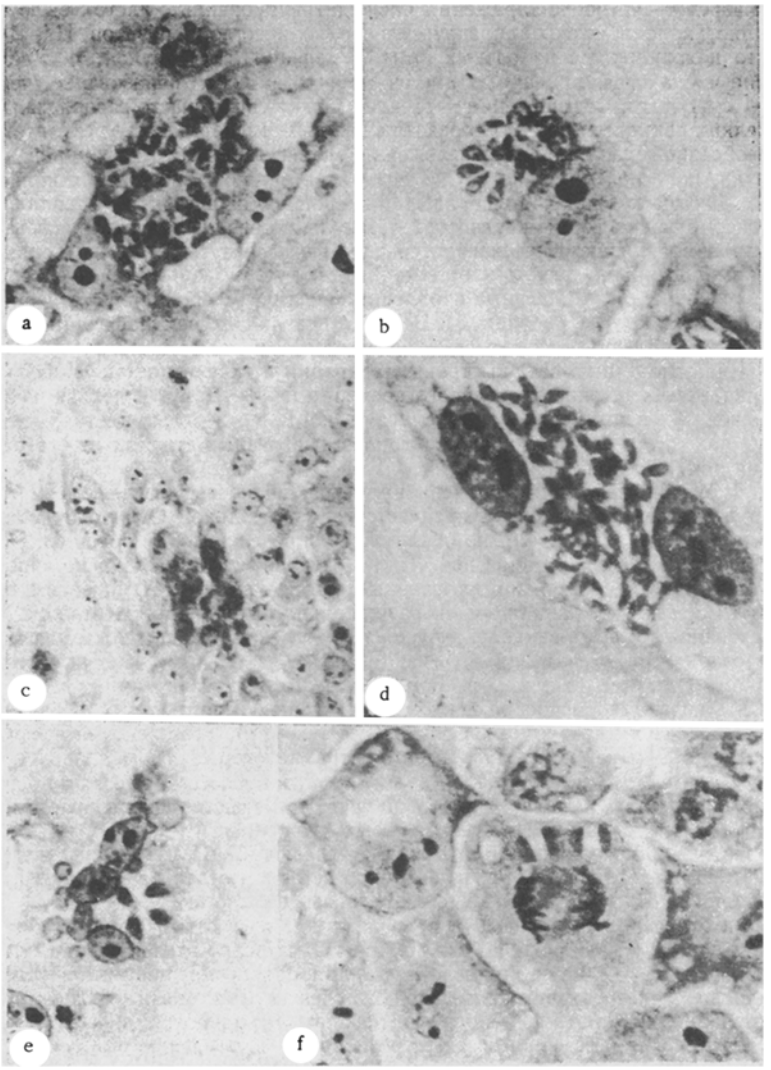

Fig. 1. Cytological changes in culture of RES cells infected with toxoplasmas: a) RES cells containing toxoplasmas at different stages of development; b) lanceolate and distended forms of toxoplasmas; c) intact RES monolayer at late stages of infection; d) cells greatly altered under the influence of parasites; e) formation of micronuclei in infected cells; f) mitosis in an infected cell.

EXPERIMENTAL RESULTS

The transplantable line of RES cells was indistinguishable in sensitivity to penetration by toxoplasmas from transplantable heteroploid cell lines studied previously [1, 7]. Characteristically, 24 h after infection the number of infected cells was small but they contained many parasites [14-16], often in different stages of division (Fig. 1a). An increase in the number of infected cells led to the formation (after 3-4 days) of foci of multiplication of the parasites. The toxoplasmas were often located in small vacuoles, with 2 or 3 parasites in each vacuole. It is not yet clear whether this was the result of isolation of the parasites by some means or other or of multiple infection of the cell. However, by 5-6 days after infection the number of intracellular toxoplasmas began to increase and degenerating forms of the parasites appeared: narrow lanceolate forms with pale cytoplasm and a pycnotic type of nucleus, and "distended" forms with a highly deformed nucleus, displaced toward the blunt end of the parasite's body (Fig. 1b). The separate agglomerations of toxoplasmas showed a tendency to stick together (Fig. 1b). In these late stages of infection, virtually no dividing toxoplasmas were present.

Infection did not cause rapid degeneration or death of the cells. The monolayer remained intact for a long time (up to 14-16 days or more after infection), including in cases when many toxoplasmas at different stages of development were present in individual cells (Fig. 1c).

For some time the culture, at first glance, appeared indistinguishable from the control despite the presence of parasites. However, a closer study showed a definite reaction to infection by the parasite. For instance, in some cells morphological changes most probably due to mechanical injury through the accumulation of large numbers of parasites could be seen: deformation of the nuclei and the formation of large vacuoles, containing toxoplasmas or not. In the later stages of infection cells of distinctive shape, showing severe changes, could be seen (Fig. 1d). Frequently widely different patterns of fragmentation of the nuclei with the formation of numerous "micronuclei" (Fig. 1e) could be observed in the infected cultures. This phenomenon has been repeatedly described in cells of cultures in certain forms of virus infection [5]. Possibly as a result of this process of nuclear fragmentation in infected cultures, binuclear cells are often found in larger numbers than in uninfected cultures.

In the late stages of cultivation (after 13-15 days) some of the cells died, the monolayer was disturbed, and separate groups of infected cells and of cells free from parasites were left on the coverslip.

The mitotic activity of the infected cultures remained high even if infection was considerable in degree. The number of mitoses fell gradually 3-6 days after infection, simultaneously with a decrease in their number in uninfected cultures, evidently on account of age degeneration of the cells (9th-11th day after the beginning of cultivation). It could even happen that infection promoted a reactive increase in mitotic activity of the culture: Whatever the case, the number of mitoses in the infected cultures was considerably higher (up to 100-80 $^{0}/_{00}$) than for the same line of cells under normal conditions (10-25 $^{0}/_{00}$) [3]. Infection of a cell did not prevent its normal vital activity: frequently mitoses were seen in cells infected with the parasites (Fig. 1f).

The problem of complete "self-sterilization" of RES cultures from parasites is not clearly understood, for besides the decrease in the number of toxoplasmas and worsening of their state (the appearance of numerous degenerating forms against the background of a well-preserved monolayer), individual cells containing normal, reproducing forms of toxoplasmas were seen in the culture. This could be evidence of possible reinfection in a given culture.

Infection of an RES culture by toxoplasmas thus does not cause severe disturbance of the vital activity of the cell and does not cause them to degenerate rapidly. The long survival of the monolayer indicates absence of any severe or advanced cytotoxic effect. However, the presence of fragmentation of the nuclei shows that reactive changes do occur, such as has been found in some other forms of harmful action on the cell [5]. Preservation of high mitotic activity, which also has been observed during the first stages of the response of the culture cell to virus infection [4], is evidently reactive in character.

The changes observed in the cell morphology are evidently chiefly the result of purely mechanical pressure of the vacuoles on the nucleus and cytoplasm, although extreme forms of disturbance of the cell morphology may arise as a result of more severe injury.

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