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RECOVERY OF SENSITIVITY OF J-41 CELLS TO COXSACKIE B3 VIRUS BY TREATMENT WITH EXOGENOUS ALKALINE PHOSPHATASE

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Investigations in the writers' laboratory have shown that the sensitivity of cells to Coxsackie B virus is largely determined by alkaline phosphatase (Enzyme Nomenclature 3.1.3.1) activity. Activity of this enzyme is sharply reduced in cells resistant to this virus [1-3, 7, 8, 10]. Alkaline phosphatase activity is unchanged in cells resistant to poliovirus; it was shown later on a different object (human–murine hybrid cells) that the sensitivity of cells to poliovirus is linked with glucose phosphate isomerase activity [9].

If loss of sensitivity of cells to particular viruses is based, inter alia, on an insufficiently high activity of certain enzyme systems, it would be logical to attempt to restore the lost sensitivity by treatment with the corresponding enzymes.

In the present study an attempt was made to restore the sensitivity of human J-41 cell cultures, which had become specifically resistant to Coxsackie B3 virus after a period of chronic infection, by introducing exogenous alkaline phosphatase.

EXPERIMENTAL METHOD

Cultures of cells of human origin were used: continuous cell line J-96, highly sensitive to Coxsackie B3 virus, and subline J-41, resistant to this virus and obtained from J-96 cells by exposure to Coxsackie B3 virus [4, 6]. After a short period of chronic infection and removal of the virus the J-41 subline was found to be specifically resistant to homologous virus and to differ from the original cells by its low alkaline phosphatase activity [1]. The cells were cultured in 50-ml flasks with strips of coverslips in medium 199 with 10% bovine

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TABLE 1. Titer of Coxsackie B3 Virus in J-96 and J-41 Cells 24 h after Infection

Material	Titer of virus, TCD_{50}/ml
J-96 cells (control)	10^8
J-41 cells (control)	10^1
J-41 cells + 50 units alkaline phosphatase*	10^3
J-41 + 250 units alkaline phosphatase	10^7

Legend. 0.01 TCD_{50} virus added per cell; J-41 cells treated with alkaline phosphatase; adsorption for 1.5 h at 37°C followed by washing cells to remove virus and alkaline phosphatase. *A further 50 units of enzyme was added to this culture after washing.

serum in the usual way. Coxsackie B3 virus was cultured in cells of continuous line PAO and titrated on highly sensitive J-96 cells. The original titer of virus used in the experiments was 10^8 TCD_{50}/ml. The number of cells in the flask at the time of addition of the virus was $2 \cdot 10^6$. The alkaline phosphatase preparation, from Sigma (USA), with an activity of 1000 units/mg protein (obtained from calf intestine), was introduced into the cell suspension or applied to the monolayer. In most experiments the growth medium was poured off 24-48 h after seeding of the cells, the cells were washed with Hanks' solution, and alkaline phosphatase was then added to some of the flasks in a dose of 2, 20, 50, 100, and 250 units in 0.2-1 ml of Hanks' solution, together with 0.2-1 ml of virus with a multiplicity of 0.01-10 TCD_{50} per cell.

The virus was adsorbed on the cells at 37°C for 1.5 h. After the end of the adsorption period some of the flasks were washed three times with Hanks' solution, 10 ml of medium 199 with 2% serum was added, and culture continued at 37°C. The enzyme was added again in the same doses to the flasks initially treated with alkaline phosphatase in doses of 2, 20, and 50 units, after the cells had been washed. Some flasks with cells were cultured without washing off the virus and alkaline phosphatase. Uninfected cells, and cells treated with alkaline phosphatase alone or with virus alone, served as the control. The cells were destroyed 24 and 48 h after infection by freezing and thawing twice. The resulting suspension was titrated on J-96 cells.

The coverslips with cells were washed in physiological saline and fixed in Bouin's mixture or in calcium-formol. Survey films were stained with hematoxylin and eosin; alkaline phosphatase was revealed by Gomori's method [5].

EXPERIMENTAL RESULTS

Resistant J-41 cells were not destroyed after infection with 10^8 TCD_{50} of Coxsackie B3 virus to $2 \cdot 10^6$ cells and the virus did not multiply in them, whereas the original sensitive cells of line J-96 all died after infection at the rate of 1 TCD_{50} virus to $2 \cdot 10^6$ cells, and the yield of virus reached 10^8 TCD_{50}/ml. Cells of the resistant line were almost entirely without alkaline phosphatase activity; high activity of this enzyme was found in the membranes and cytoplasm of the sensitive J-96 cells. Alkaline phosphatase, added to a suspension of J-41 cells in a dose of 225 units, did not prevent the formation of a cell layer, but subsequent infection of the monolayer with virus led neither to death of the cells nor to reproduction of the virus. No alkaline phosphatase activity could be demonstrated by Gomori's method in J-41 cells 24 h after treatment with the enzyme. Meanwhile, the addition of alkaline phosphatase simultaneously with virus led to reproduction of the virus; the level of replication of the virus depended on the quantity of enzyme added (Table 1).

The intensity of the cytopathic changes in the resistant J-41 cultures treated with alkaline phosphatase depended both on the dose of enzyme and on the multiplicity of infection. If the cells were treated with 250 units of alkaline phosphatase in a volume of 0.2 ml with simultaneous addition of virus in a high concentration (1-10 TCD_{50} per cell), the cell monolayer was completely destroyed after 24 h, irrespective of whether the virus and enzyme were washed off or not after the period of adsorption of virus. The titer of virus in these experiments was $10^{6.5}$-10^7 TCD_{50}/ml. Control sensitive J-96 cells also died: the titer of virus was 10^8 TCD_{50}/ml. With a low multiplicity of infection (0.01 TCD_{50} per cell) and under similar experimental conditions, a cytopathic effect did not develop after 24 h in the resistant cultures; single foci of specific cell destruction were seen in sensitive J-96 cultures. The titer of virus determined after freezing and thawing of the cells twice was 10^7 TCD_{50}/ml for the resistant and 10^8 TCD_{50}/ml for the sensitive cells.

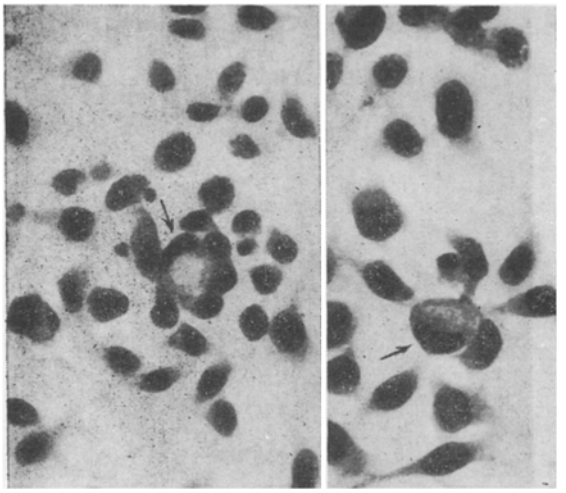

Fig. 1. J-41 cells 48 h after addition of 100 units of alkaline phosphatase and infection with Coxsackie B3 virus in a dose of 10 TCD_{50} per cell. Separate cells with changes characteristic of the cytopathogenic action of Coxsackie B3 virus (arrows). Phagocytosis of dying cells. Hematoxylin and eosin; 100×.

Addition of 100 or 50 units of alkaline phosphatase did not give clear results in all experiments, as regards either morphological changes in the cells or reproduction of the virus. Addition of 100 units of enzyme, combined with high multiplicity of infection (10 TCD_{50} per cell), led to the appearance of single cells in the resistant J-41 culture with morphological changes characteristic of the cytopathogenic effect of Coxsackie B3 virus (Fig. 1). However, no statistically significant multiplication of the virus could be observed in these experiments. With a low multiplicity of infection (0.01 TCD_{50} per cell), however, the addition of even 50 units of enzyme in the period of adsorption of virus followed by addition of a further 50 units after washing of the cells led to the appearance of single foci of specific cell destruction in the resistant J-41 cultures: the titer of virus was 10^3 TCD_{50}/ml (Table 1).

Addition of smaller doses of the enzyme led neither to multiplication of the virus nor to the development of specific morphological changes in the resistant J-41 cells.

The addition of homologous virus together with an adequate dose of exogenous alkaline phosphatase to a culture of J-41 cells resistant to Coxsackie B3 virus thus leads to propagation of the virus and to the development of a cytopathogenic effect, in the form either of total destruction of the monolayer or of death of single cells, with the appearance of morphological changes in them characteristic of the cytopathogenic effect of Coxsackie B3 virus. Consequently, there is reason to consider that the fall in alkaline phosphatase activity is the factor which determines the specific resistance of J-41 cells to Coxsackie B3 virus. This resistance can be overcome by addition of exogenous alkaline phosphatase.

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