INHIBITORY EFFECT OF HOMOLOGOUS ANTIVIRAL ANTIBODIES ON VIRAL INJURY TO CELLS

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To obtain chronic virus infections in cell cultures the addition of homologous antiviral immune sera to the culture medium is widely used. It is generally accepted that antiviral antibodies fix and neutralize any virus produced. This leads to blocking of the transmission of infection through the culture fluid and prevents infection of new cells. Transmission of infection from cell to cell by direct contact is accepted, and this leads to enlargement of the primary foci of infected cells. However, models of cell cultures chronically infected by many viruses have been described, in the culture fluid of which, despite the addition of immune serum, infectious virus constantly accumulates; this contradicts the views given above on blocking of virus and prevention of infection of new cells [1, 2]. This suggests the existence of another mechanism responsible for the development and maintenance of chronic virus infection in the presence of antiviral antibodies.

The object of this investigation was to examine more closely the effect of antiviral antibodies on the development of chronic virus infection.

EXPERIMENTAL METHOD

A continuous monolayer culture of Chinese hamster 431 cells, obtained from the Institute of Molecular Biology, Academy of Sciences of the USSR, was grown on Eagle's medium with 10% bovine serum and was infected once with Sindbis virus (strain EgAn-339) in the form of a 10% suspension of mouse brain with a titer of $10^{7.5}$ TCD_{50}/ml, kept in a lyophilized state at −20°C. The titers of virus were determined according to their cytopathic action on a culture of chick embryonic cells. The dose for infection was 5-15 TCD_{50} per cell. Under these conditions a culture infected with Sindbis virus developed an acute infection with active reproduction of virus (the titers reached 7-8 log TCD_{50}/ml) and with a well-defined cytopathic action (intensive accumulation of surface and intracellular virus antigens, rounding of the cells and their separation from the glass) in the course of 24-48 h [4].

EXPERIMENTAL RESULTS

In an uninfected culture most cells in a well-formed monolayer were polygonal in shape; about 3% were rounded, probably during mitosis, and were intensively stained, so that their structure could not be clearly distinguished, and about 1% of cells were degenerating. The number of rounded cells increased particularly sharply (to 70-80%) 24 h after infection with Sindbis virus as a result of the development of cytopathic changes (Table 1).

Addition of 0.5% immune serum against Sindbis virus* to the culture medium after adsorption of the virus by the monolayer culture completely prevented the development of cytopathic changes. Extracellular virus accumulated constantly in the culture medium in titers of up to 3.7 log TCD_{50}/ml. The heterologous immune serum had no such effect.

Replacement of the culture medium containing homologous immune serum 24 h after infection by medium without the immune serum or with heterologous serum caused activation of the in-

*The immune serum had a titer of 1:640-1:1280 in the hemagglutination inhibition test against eight antigenic units.

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TABLE 1. Characteristics of Culture of Chinese Hamster Cells Grown on Medium with or without Immune Serum, 24 h after Infection with Sindbis Virus

No.	Addition of immune serum	Fraction of cells, %		
		polygonal	rounded	degenerating
	Uninfected culture			
1	0	96±2,7	3,2±2,4	1,2±0,8
2	0.5% to Sindbis virus	96,4±2,6	3,2±1,2	0,5±0,3
	Infected culture			
3	0	20,3±6,7	73±5,8	9,5±3,6
4	0.5% to vesicular stomatitis virus	19,2±11	67,8±5	11,3±5,8
5	0.5% to Sindbis virus	95,7±2,1	3,4±1,3	1,2±0,7

Legend. Differences between groups 1, 2, 5, and 3, 4 significant for all parameters studied ($P < 0.01$).

TABLE 2. Characteristics of Culture of Chinese Hamster Cells, Infected with Sindbis Virus, and Grown in Medium with Homologous Antiviral Serum

Culture		Fraction of cells, %		
passage after infection	time of examination after transplantation	polygonal	rounded	degenerating
3rd	24	83,9±4	8,1±1,6	3,2±2,5
3rd	48	89,4±3,3	6,7±3,4	2±1,3
5th	24	94±1,1	5,6±1,4	0,6±0,5
Uninfected	48	93,2±1,5	5,6±1,2	0,9±0,4

fection. It was found that 24 h after replacement in this manner 49.5 ± 1.3% of the cells were polygonal, 43 ± 12% were rounded and 7.8 ± 2.8% of cells were degenerating. In the uninfected control culture at the same time 94.3 ± 2.2% of cells were polygonal, 3.9 ± 2.2% were rounded, and 2 ± 1.7% degenerating.

Culture of Chinese hamster cells infected with Sindbis virus in medium with the addition of 0.5% antiviral homologous immune serum led to the development of chronic infection with constant accumulation of extracellular virus in titers of from 1 to 3.9 log TCD_{50}/ml. No cytopathic changes were present in the culture. The characteristics of the cell composition of these cultures are given in Table 2. One of the chronically infected cultures was examined in the course of 1.5 months and 19 passages (Fig. 1). In five samples of culture fluid collected from this culture at the 2nd, 4th, 7th, and 9th passages, attempts were made to find interferon, which would have inactivated Sindbis virus, by acidification to pH 2.0. The material was titrated for interferon in tubs with a culture of Chinese hamster cells on the basis of their protection against the cytopathic action of vesicular stomatitis virus. No interferon was found.

Both intracellular and surface (localized in the plasmalemma) virus antigens (Fig. 2, a and b) were detected by the method of fluorescent antibodies and immune cytolysis in cells of the infected cultures. The addition of antiviral serum to the medium led to a considerable decrease in the content of viral antigens in the cells (Fig. 2, c and d; Table 3). Antiviral antibodies were adsorbed on the plasmalemma of the cells and usually were revealed as granules on the surface of cells grown in medium with immune serum, or as rims in the case of acute infection (Fig. 2). The addition of less diluted immune serum against Sindbis virus (1:3) and complement to the infected culture for the immune cytolysis test increased the number of lysed cells. This was evidence that free antigenic determinants were present on the surface of the infected cells (Table 3). Antigens were found on all types of cells, including those dividing by mitosis, and polyploid and multinuclear cells. The number of antigen-positive cells detected by the fluorescent antibodies method was several times greater than the number of lysed cells. Similar relationships were observed previously in a study of the principles governing virus-specific immune cytolysis [3].

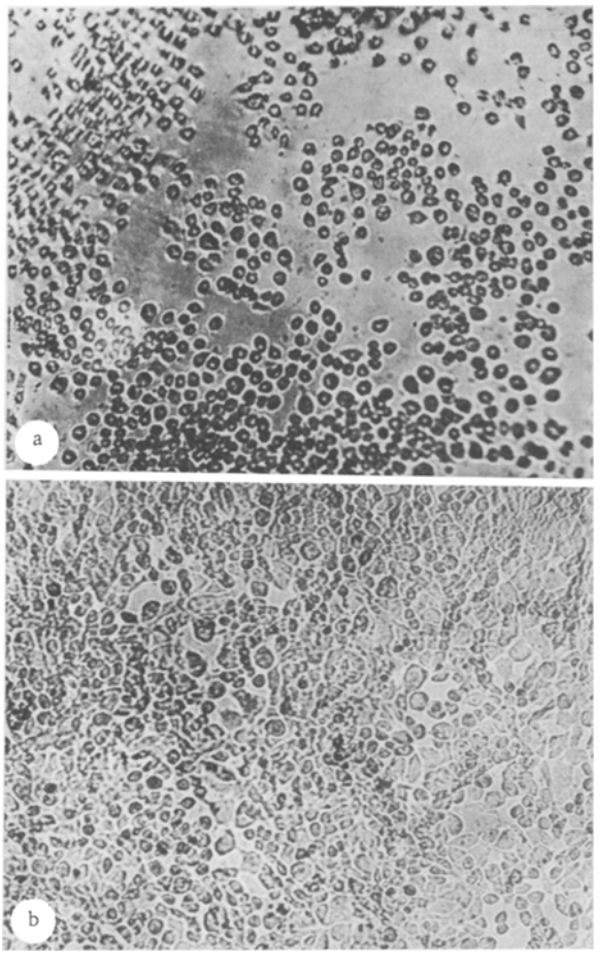

Fig. 1. Cytopathic changes in culture of Chinese hamster cells infected with Sindbis virus: a) 3rd passage; 18 h of culture on medium without immune serum against Sindbis virus; b) 18th passage; medium with 0.5% immune serum against Sindbis virus. Vital preparations, 100 ×.

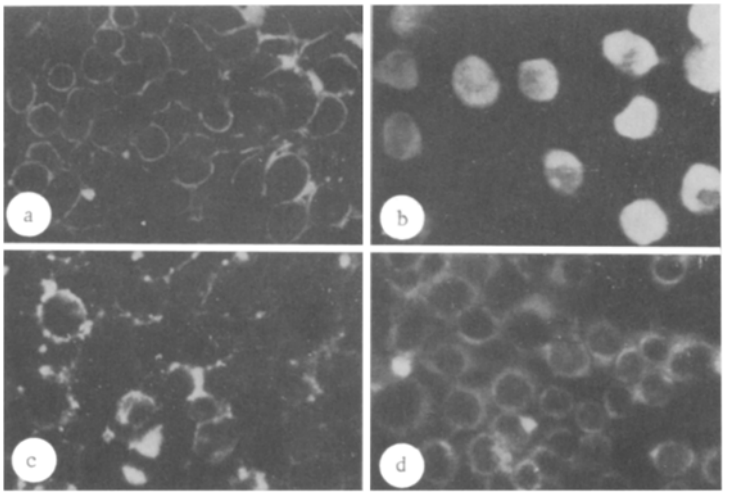

Fig. 2. Immunofluorescence detection of surface (a, c) and intracellular (b, d) antigens of Sindbis virus. Culture of Chinese hamster cells with acute (a, b) and chronic (c, d) infection with Sindbis virus. Indirect fluorescence antibodies method. 200 and 400 ×.

TABLE 3. Immune Cytolysis in Culture of Chinese Hamster Cells Infected with Sindbis Virus

No.	Passage after infection of culture	Time of examination after transplantation, h	Number of lysed cells (in %) after addition of	
			complement (A)	immune serum against Sindbis virus and complement (B)
1	Uninfected	48	0	0
2	24 h after primary infection	48	0	45±14
3	3rd	24	7±4,6	15,2±9
4	3rd	48	n.d	12,3±11
5	5th	24	n.d	4,4±3,6

Legend. 1) n.d. — Not determined. 2) Difference in number of lysed cells between 2B and 3B-5B and 3A and 3B significant ($P < 0.01$).

Inhibition of virus injury to the cells which was observed cannot be explained by blocking of transmission of infection by antibodies through the culture fluid and prevention of infection of new cells. The most likely explanation is blocking of the function of the virus genome in the affected cell under the influence of homologous antibodies bound with virus-inducing antigens in the plasmalemma of the cells.

LITERATURE CITED

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