PERMEABILITY OF NORMAL AND VIRUS-INFECTED CELLS TO PROTEINS OF THE γ-GLOBULIN FRACTIONS OF NORMAL AND IMMUNE SERA

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The permeability of cells to the serum γ-globulins and, in particular, to antibodies is an unsolved problem giving rise to many conflicting opinions. Most observations tend to show that the action of virus-neutralizing antibodies is limited to extracellular virus and does not extend to virus located within the cell [1, 4, 9-16]. Only a few investigators have mentioned the possibility that antibodies may act on intracellular virus [5-8].

This paper describes the results of cytochemical and virological investigations of the permeability of normal and virus-infected cells to proteins of the γ-globulin fractions of normal and immune sera.

EXPERIMENTAL METHOD

Virus of vesicular stomatitis (VS), strain New Jersey, was used in the experiments in the form of the culture fluid of infected chick embryonic fibroblasts. Usually a monolayer culture of chick fibroblasts obtained from trypsinized chick embryos was used, grown on Gey's medium with 5% calf serum. Primary monolayer cultures of chick embryonic kidneys, mouse embryonic fibroblasts, and the kidneys, lungs, spleen, and macrophages of adult albino mice, human embryonic kidneys, lungs, and fibroblasts, and the kidneys of guinea pigs and monkeys were also used. Besides the above, transplantable cultures of human embryonic lungs, the kidneys of the guinea pig, the monkey, and the pig embryo, HeLa, L, Detroit-6, and HEp-2 cells, and human amniotic cells were used in the experiments.

To determine whether γ-globulin could pass into the cells of the tissue cultures, labeled and untreated serum γ-globulins were studied.

To study the penetration of labeled γ-globulins into the cells, normal serum γ-globulins of the rabbit and chicken, immune rabbit γ-globulins against VS virus, and rabbit anti-chicken γ-globulin were used. The γ-globulin fraction of the sera was obtained by means of rivanol* or by precipitation with sodium sulfate. The globulins (2%) were conjugated with fluorescein isothicoyanate at the rate of 2 mg of dye per 100 mg of protein. The labeled proteins were sterilized by passage through a Seitz filter, poured into ampules, lyophilized, and stored in the dark at 4°. The dried conjugate was made up with bidistilled water immediately before the experiment and introduced into the tissue culture grown in tubes on cover slips in a dose so that the final dilution of protein in the nutrient medium was 1-3 mg/ml. The nutrient medium was a mixture of equal volumes of medium No. 199 and Earle's medium. The cultures were incubated with labeled γ-globulins for 1-48 h at 36°. At the end of each period the cells were washed several times with phosphate buffer or medium No. 199, dried in air, and fixed for 10 min with ethyl alcohol.

To study the penetration of untreated γ-globulins into the cells, the direct method of Coons was also used. Cells grown on cover slips were washed several times with phosphate buffer or medium No. 199, dried in air, and fixed for 10 min in 98° ethyl alcohol. They were then stained with a specific chicken anti-rabbit γ-globulin in a moist chamber for 30 min and washed off for 10 min with buffered physiological saline, pH 7.2. All the above procedures were carried out at room temperature. The control consisted

*2-ethoxy-6, 9-diaminoacridine

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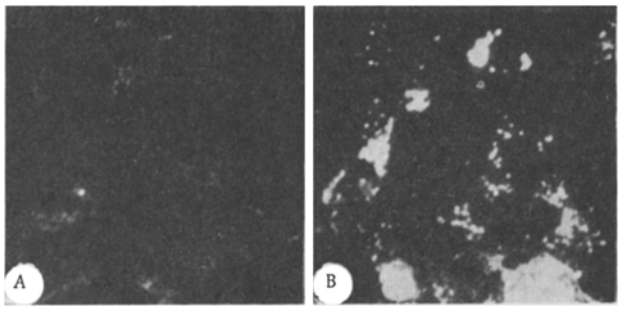

Fig. 1

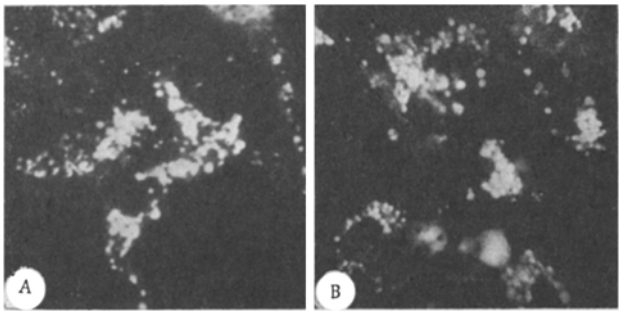

Fig. 2

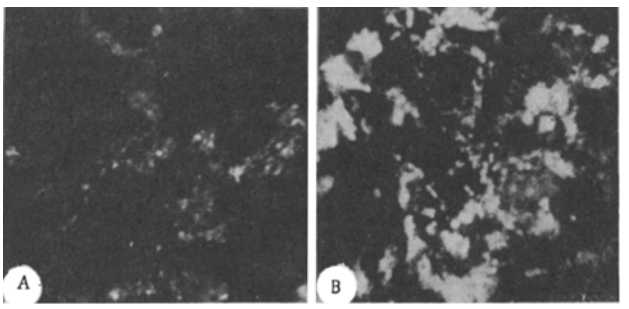

Fig. 3

Fig. 1. Normal rabbit labeled γ-globulin in normal chick fibroblasts: A) 2 h after addition of the culture; B) 8 h after addition of the culture. Objective 90×, ocular 5×.

Fig. 2. Labeled rabbit γ-globulin immune to vesicular stomatitis virus, in normal (A) and virus-infected (B) chick fibroblasts 8 h after addition of protein to the culture (12 h after infection of the culture with VS virus in a dose of 6 log ID_{50} /ml). Objective 90×, ocular 5×.

Fig. 3. Untreated rabbit γ-globulin, immune to VS virus, in chick fibroblasts. Treatment with antiglobulin luminescent serum: A) 3 h after addition of protein to culture; B) 12 h after addition of protein to culture. Objective 90×, ocular 5×.

of tissue cultures treated with "exhausted" conjugate, cultures incubated with heterologous (human) γ-globulin and then treated with specific antiglobulin conjugate, and also normal cultures treated after fixation with labeled normal or immune γ-globulin.

Luminescence microscopy was carried out in incident long-wave ultraviolet light by means of the ML-1 luminescence microscope with filters FS = 1 + SZS = 7, with objectives of 40, 70 (water immersion), and 90× (oil immersion) and oculars of 4, 5, and 10×. To determine the localization of the labeled globulin in the cells more accurately, in separate experiments observations were made with phase contrast.

Photographs were taken on RF-3 film (180 state standard units) with an exposure of between 20 and 40 sec.

The method of determination of the content of intracellular virus was described previously [1].

EXPERIMENTAL RESULTS

Permeability of the cells to labeled γ-globulins. The investigations showed that the normal labeled proteins of the γ-globulin fraction of the rabbits's and chicken's serum and also the immune globulins of the rabbits against VS virus or against chicken γ-globulin penetrated into the chick fibroblasts. In individual cells of the culture 1-2 h after addition of the protein to the nutrient medium, tiny luminescent granules appeared around the nuclei, gradually increasing in number and size. From 6 to 10 h later nearly all the cells gave a bright and clear fluorescence in the form of large and small luminescent granules distributed in the perinuclear zone, and sometimes throughout the cytoplasm (Fig. 1). Subsequently the intensity of the fluroescence increased slightly, as was shown by an increase in the size and number of the granules in the cytoplasm.

The normal and immune γ-globulins penetrated equally into the normal and the infected cells (Fig. 2).

The study of the permeability of the cells of the different monolayer cultures to labeled normal and immune (to VS virus) rabbit γ-globulins demonstrated the ability of the cells of several cultures to absorb labeled proteins. This property was most marked in the primary cultures of fibroblasts and macrophages, and it was very weak in the primary epithelial and the transplanted ephithelial and fibroblast cultures (see Table). The exception was the transplantable culture of fibroblasts from the human embryonic lungs, in which numerous small luminescent granules were found.

Permeability of the cells to untreated γ-globulins. The study of the penetration of the proteins of the γ-globulin fraction into the cells by the direct Coons' method confirmed the results described above. The normal and immune (to VS virus) rabbit globulin penetrated into the cells of the chick fibroblast culture

Penetration of Labeled Rabbit γ-Globulin into Cells of Various Tissue Cultures

Type of culture	Nature of culture	Presence or absence of luminescent granules in cells 24 h after addition of labeled protein
Primary	Chick embryonic fibroblasts	+++
	Kidneys of chick embryo	+
	Fibrobasts of mouse embryo	+++
	Macrophages of adult mice	+++
	Lungs	+++
	Kidneys	+
	Spleen	+++
	Lungs of human embryo	+++
	Fibroblasts	+++
	Kidneys	+
	Kidneys of guinea pigs	+
	Kidneys of adult monkey	+
Transplantable	Lungs of human embryo	+++
	HeLa	−
	Detroit-6	−
	HEp-2	+
	Human amniotic cells	+
	Kidneys of a guinea pig	+
	Kidneys of a monkey	+
	Kidneys of pig embryo	+
	L cells	+

Legend: +++ luminescent granules present in nearly every cell; + luminescent granules present in solitary cells; - no luminescent granules in the cells.

within 1 h after its addition to the culture. After 2-4 h this antigen was found in nearly all the cells, which gave a bright yellow-green fluorescence, and the absorption of the antigen was maximal after 12-18 h (Fig. 3). The protein which had penetrated into the cells was distributed in granules or diffusely. In contrast to the experiments with labeled proteins, in these investigations penetration of the globulin was observed not only into the cytoplasm, but also into the nucleus, although the degree of fluorescence of the nucleus was only slight. Similar results were obtained in the experiments with the cultures of human and mouse fibroblasts.

Reproduction of VS virus in cells incubated in the presence of antibodies. The results described above demonstrated that normal and immune γ-globulins have the power to penetrate into cells. To study the action of the penetrating immune serum proteins, possibly containing antibodies as well, on intracellular virus the dynamics of virus reproduction was examined in cells incubated in the presence of antibodies.

The curves of intracellular reproduction of VS virus in the tissue culture of chick fibroblasts infected with a massive dose of the virus (6.4 log ID_{50}/ml) in the presence of labled rabbit γ-globulin immune to VS virus or of unlabeled immune γ-globulin were essentially indistinguishable. The antibodies present in the nutrient medium had no visible effect on the intracellular virus.

In neutralization of a certain part of the virus took place inside the infected cells, this change in virus activity could not be detected in the conditions of infection of the culture with a massive dose of virus, essential for the instantaneous infection of the mass of cells in the tissue culture.

To obtain a more precise answer to this question, the course of reproduction of VS virus was compared in two different cultures, one of which (chick embryonic fibroblasts) was found by luminescence microscopy to be permeable, while the other (Detroit-6) was impermeable to the immune globulins of the rabbit serum. Infection of the tissue cultures in these experiments was with small doses of virus (10-1000 ID/ml). In these conditions, likewise, no difference was discerned between the reproduction of the virus in the two cultures, despite the difference in their permeability to immune globulins. Degeneration of the infected monolayer was inhibited in both cultures in the presence of antibodies. Removal of the antibodies from the nutrient medium led to appearance of the virus in the culture fluid and to rapid degeneration of the cells.

In the control flasks incubated without antibodies, the content of intracellular virus was much higher than in the presence of antibodies. Twenty hours after infection, marked cytopathic changes developed; they progressed in the course of incubation and led to total destruction of the culture after 36-48 h.

The sharp decrease in the content of virus, compared with the controls, in the cells infected by small doses of virus and incubated in the presence of antibodies may be attributed to the inability of the virus to spread freely from the infected cells to the uninfected. Antibodies were not present in the control tissue cultures, and the virus circulated unhindered between the infected and the healthy cells.

The results described in this paper demonstrate the ability of the proteins of the γ-globulin fraction of normal and immune sera to penetrate into cells. Since this had no visible effect on the intracellular reproduction of the virus, the question of the permeability of the cells to antibodies remains unanswered. To obtain an answer, experiments must be carried out with pure antibodies free from all contamination by other proteins.

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