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MECHANISM OF RECEPTION OF INFLUENZA VIRUSES BY SOMATIC TISSUE CELLS

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KEY WORDS: influenza viruses; low-density lipoproteins; receptor-induced endocytosis.

Reception of virus particles by tissue cells takes place through high-affinity sites (receptors) located on the cell membrane [10].

A theory of receptor-mediated uptake of low-density lipoproteins (LDL) by tissue cells has recently been developed in detail [5, 6]. Analysis of the mechanisms or receptor uptake of virus particles and LDL indicates a large number of identical components of these processes. In both cases reception of virus and lipoprotein particles takes place through high-affinity sites, in three consecutive stages: binding of particles by receptors (internalization), endocytosis in the composition of coated vesicles, and LDL metabolism proper, or reproduction if the subject is virus.

LDL, whose composition includes large quantities of cholesterol (Chs) provide the tissue cells with this metabolite, which they need in order to construct their outer and inner membranes. The supply of Chs to the cell by the mechanism described above is therefore essential for the normal physiological activity of the tissue cells. It can accordingly be postulated that virus particles can penetrate into the cell through the same high-affinity sites as for LDL on the surface of the outer cell membranes. This hypothesis is based on the improbability that nature would create special receptors to transport virus particles inside the cell, which would ultimately lead to death of the cell.

The aim of the present investigation was to compare the structure of cellular receptors responsible for transport of LDL and virus particles, by comparing their immunological properties.

EXPERIMENTAL METHOD

Experiments were carried out on a tissue culture of human embryonic lung fibroblasts (HELF) at the 10th passage. Experiments on the cells were carried out in plastic Petri dishes 16 mm in diameter ("Falcon," USA), using the GPI-01 CO_2 inducer. The fibroblasts were used in the experiments after formation of a monolayer in 10% bovine serum in Eagle's medium; cells also were grown on Eagle's medium containing penicillin (100 U/ml), streptomycin (100 μg/ml), and nonlipoprotein serum proteins (bottom proteins) (d > 1.250 g/ml NaBr), isolated by ultracentrifugation by the method in [7], in a concentration of 5 mg/ml. Influenza virus strain A/PR8/34 was added to the cell culture in equal amounts and incubated in a CO_2 incubator at 37°C, and in an atmosphere containing 5% CO_2 for 5 h. The culture medium was then poured off, the cell culture was carefully washed with Eagle's medium containing antibiotics and bottom proteins in order to remove virus particles not bound with the cells, media of the above-mentioned composition were again added, and incubation in the

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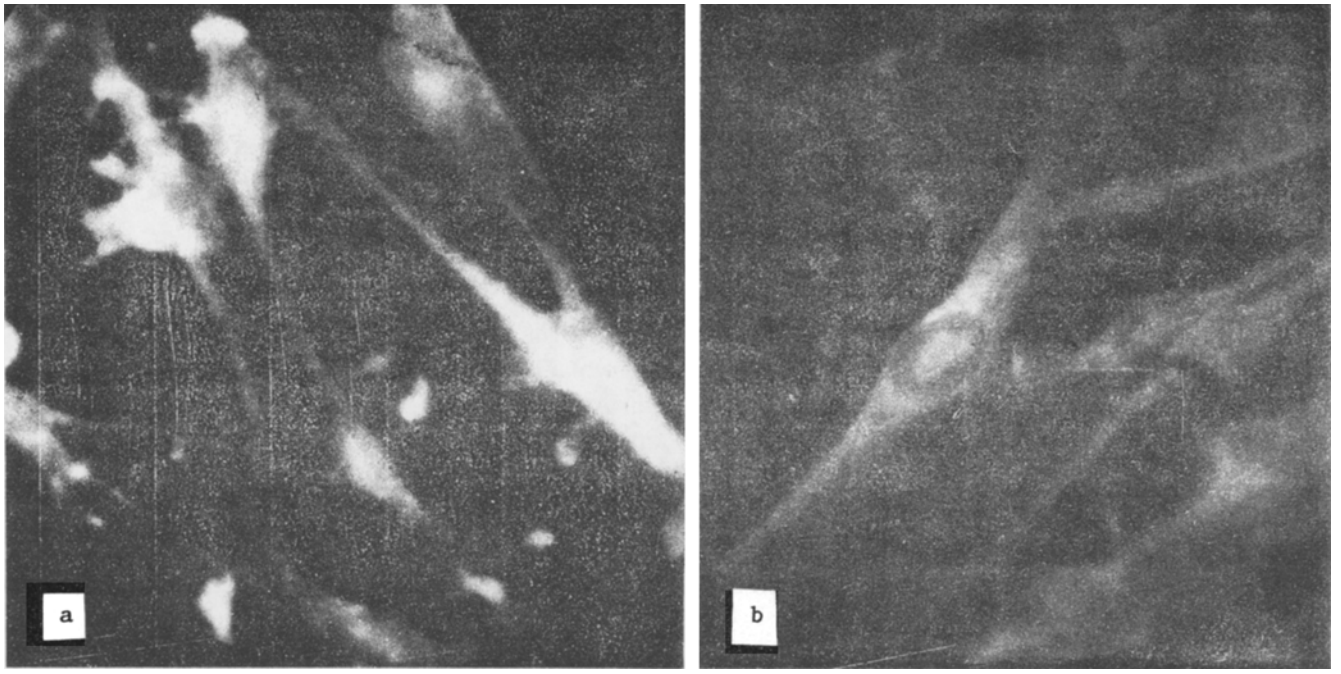

Fig. 1. Interaction of HELF with influenza virus strain A/PR8/34. a) Fibroblasts grown with addition of LDL to culture medium; b) fibroblasts grown with addition of bottom proteins, not containing LDL, to culture medium.

CO_2-incubator was continued for 48 h. The influenza virus antigen was identified on the surface of the cells by Coons' method, using fluorescent type-specific antibodies, conjugated with fluorescein isothiocyanate, and also in the reaction of neutralization of infectious activity on 11-day chick embryos by the following method. A cell suspension for titration was taken from the Petri dishes after freezing and thawing, and was used in dilutions of 10^{-1}, 10^{-2}, 10^{-3}, and so on. Two embryos were infected with each dilution and incubated in a water thermostat at 37°C for 48 h. The viruses were identified in the hemagglutination test (HAT) and the infectious titer of the virus was determined by the method of Reed and Muench and expressed in embryonic infectious doses (EID_{50}/0.2 ml).

LDL (1.040-1.053 g/ml NaBr) were isolated by ultracentrifugation [7] from normal human blood. Apoprotein B (apo-B) was isolated by the method in [4]. Antiserum to apo-B was obtained by immunization of rabbits with the isolated protein [3]. Immune activity of the rabbit sera and also the complementary activity of these sera with viral antigen were studied by the precipitation test in 1% agar gel [9] and in the complement fixation test (CFT) [1]. The protein concentration in the samples was determined by Lowry's method [8].

The lipid (Chs) concentration was studied in the blood of CBA mice (10 intact and 10 infected with Bethesda influenza virus) on an AA-2 automatic analyzer ("Technicon," USA), using international standardization of the blood lipid investigation [2].

The study of binding and uptake of virus particles by HELF, previously incubated in medium containing 10% bovine serum, and which included in its composition LDL, and also cells preincubated in medium not containing LDL, revealed the following general conclusions. As will be clear from Fig. 1, fluorescence of cells preincubated in medium not containing LDL was considerably stronger than in a culture grown on medium containing 10% bovine serum, This fact points to the possibility of competition between apo-B-containing LDL and virus particles for receptors on plasma cells. This fact is evidence that receptors for native LDL on cells grown in medium containing bovine serum are blocked by apo-B-containing LDL in the composition of bovine serum, whereas in a culture of cells whose growth medium did not contain LDL, the intensity of uptake of virus particles was greater. Similar results were obtained by the neutralization of infectious activity of the virus test on 11-day chick embryos.

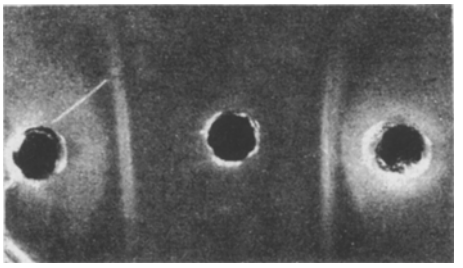

Fig. 2. Precipitation test in 1% agar gel on antisera to influenza virus and apo-B-protein of LDL with hemagglutinin of influenza virus strain A/PR8/34. On right – antiserum to virus; on left – antiserum to apo-B-protein of LDL.

TABLE 1. Comparison of Immunologic Properties of Anti-Apo-B and Antiserum to Texas Influenza Virus using the CFT

Dilution	Adsorbed influenza vaccine			Adsorbed Texas influenza virus		
	Apo-B serum	Texas serum	Control serum	Apo-B serum	Texas serum	Control serum
1:10	128	256	±	128	1028	—
1:20	128	256	—	64	1028	—
1:40	64	128	—	64	1028	—
1:80	16	8	—	64	256—512	—
1:160	4	4	—	64	256—512	—
Control activity	2	—	—	—	—	—

Thus the addition of 10% bovine serum containing LDL to the culture medium led to a sharp decrease in infectious activity of the A/PR8/34 virus. This fact also indicates that far fewer virus particles penetrated into the cells of an HELF culture grown on medium containing LDL, for the cell receptors located on the outer membrane were blocked by apo-B-containing lipoproteins. This did not happen with cells grown on incubation medium not containing LDL. It can thus be tentatively suggested that apo-B-containing lipoproteins and virus particles have the same high-affinity sites (receptors) on their outer cell membrane, performing endocytosis of these particles into the cell. If this hypothesis is true, immunologic similarity must exist between the receptor protein for apo-B-containing lipoproteins and the hemagglutinin of influenza virus. In fact, antibodies to apo-B-protein of LDL are copies of regions through which lipoproteins penetrate into the cells, and in the case of interaction of these antibodies with influenza virus hemagglutinin it can be considered that both sets of particles have common antigenic determinants which are involved in the realization of stage I of specific endocytosis, namely internalization. As our investigations showed (Fig. 2) the hemagglutinin of influenza virus A/PR8/34 forms a precipitation arc with antibodies to apo-B-protein and also with antibodies obtained to that virus (vaccine), confirming the hypothesis put forward above. Results reflecting the same immunologic trend also were obtained by the CFT (Table 1).

The study of the blood Chs level in mice infected with the Bethesda strain of influenza virus gave the following results.

During the 1st day after the beginning of the experiment the serum Chs level of the experimental animals rose (by 10%), to reach a maximum on the 2nd day, and it remained at that level until the 5th day (20%), suggesting an increase in the concentration of the LDL fraction in the blood of the mice. This fact may also be evidence in support of the view that influenza viruses and LDL compete for common high-affinity sites located on the outer cell membrane.

It can thus be tentatively suggested that the influenza viruses of strains A/PR8/34, Texas, and Bethesda, which we studied, can penetrate tissue cells at sites of high affinity, intended by nature for LDL. It is perfectly probable that reception of many other virus particles also takes place with the aid of the mechanism described above. Nevertheless it must be

emphasized that the data given in this paper provide no basis for the conclusion that virus particles can enter the cell only through receptors for LDL. It is perfectly probable that viruses may also have specific receptors through which they can penetrate into tissue cells.

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CHOICE OF ADEQUATE METHOD OF DETOXICATION AND IMMUNOCORRECTION IN EXPERIMENTAL DESTRUCTIVE PANCREATIS

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KEY WORDS: experimental pancreatitis; detoxication; immunocorrection.

One of the most difficult problems in emergency abdominal surgery is that of acute pancreatitis, which is usually accompanied by a marked toxic syndrome (pancreatogenic toxemia). This syndrome develops in the initial period of the disease [9], and if detoxication therapy is not instituted during this period, a vicious circle develops. The barrier inhibitory mechanisms are disturbed, the local pathological process is intensified, and progresses under the influence of generalized microcirculatory disturbances and a syndrome of disseminated intravascular clotting. Only timely differential detoxication treatment can prevent subsequent progression of the pathological process in its local and general manifestations. In recent years, besides traditional methods of treatment, much attention has been paid to the use of detoxication methods: hemoperfusion (HP), plasmapheresis (PPh), ultraviolet irradiation (UVI) of autologous blood, and xenosorption (XS). However, these methods are often used unsystematically, without any attempt at differentiation for scientifically based choice, disregarding the particular features of the course of pancreatogenic toxemia, manifested as massive release of activated pancreatic enzymes and proteolysis products into the portal circulation, and also by immunologic disturbances in the body.

The aim of this investigation was to develop an effective, pathogenetically based method of detoxication and immunocorrection in destructive pancreatitis with a marked toxemia syndrome.

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