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Fatty acid acylated antibodies against virus suppress its reproduction in cells

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A method for suppression of virus reproduction in cells using fatty acylated antiviral antibodies, which in contrast to non-modified antibodies are capable of intracellular penetration, has been suggested. The addition of stearoylated antiviral antibodies to influenza A/Chili virus-infected cells causes a 100-fold suppression of virus reproduction. Non-modified antibodies do not produce any effect on virus reproduction.

Antibody; Fatty acylation; Virus suppression; Influenza

1. INTRODUCTION

Modern biotechnology uses highly effective means for the production of antibodies against antigen determinants of different viruses. This fact ensures the rapid progress in the development of methods for the diagnoses and investigation of viral diseases [1,2]. This raises the question: why are antiviral antibodies not used practically [2] for the therapy of such diseases and, in particular, for the suppression of virus reproduction.

It is known that antibodies against virus can prevent the infection of cells by this virus [3]. However, the same antibodies do not affect the development of infection in already infected cells, since they cannot penetrate into them and block intracellular reproduction of the virus [4].

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Abbreviations: Hepes, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid sodium salt; Aerosol OT, bis-(2-ethylhexyl)-sulfosuccinate sodium salt; PFU, plaque forming units; HAU, hemagglutination unit

An effective method for imparting transmembrane properties to water-soluble proteins has been developed recently [5-7]. For this purpose, a lipid 'anchor' (fatty acid residue) is covalently attached to the protein molecule. As a result, hydrophobized proteins retain their functional activity, acquiring an ability for translocation across lipid membranes and penetration into infected cells.

In the present work we have used influenza A/Chili/1/83(H1N1) virus to study possibilities of suppression of virus reproduction by antiviral antibodies, artificially hydrophobized with stearic acid residues.

2. MATERIALS AND METHODS

2.1. *Cells*

MDCK cells were kindly presented by the Laboratory of Cell Cultures (Institute of Virology, USSR Academy of Medical Sciences). Cells were grown as a monolayer stationary culture in 199 medium, containing 10 mM Hepes, 0.075% sodium bicarbonate, 10% fetal calf serum and 200 μg/ml gentamycin.

2.2. *Antibodies and their modification*

To obtain polyclonal antibodies against influenza A/Chili virus, mice were immunized according to a standard technique

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[8]. Modification of antibodies by stearoyl chloride was carried out in the system of reversed micelles of Aerosol OT in octane by the method detailed in [5,9]. 1.5 ml of 50 mM solution of antibodies in 0.1 M borate buffer, pH 9.5, were added to 10 ml of 0.3 M Aerosol OT solution (Merck) in octane. The system was intensively shaked at 20°C for 1–2 min to achieve optical density, and then 1.5 ml of 0.25 mM stearoyl chloride solution in octane were added to it. Stearoyl chloride was obtained by treating stearic acid (Sigma) with thionyl chloride and purified by distillation in vacuum. After 2 h the protein was precipitated with 30 ml of cold (0°C) acetone. The precipitate formed was then removed by centrifugation and thoroughly washed five times with 30 ml of cold (4°C) acetone. The residual acetone was removed using a rotor evaporator. The modified antibodies were purified by gel filtration on Biogel P2.

The modification degree was determined as described [9] using [^{3}H]stearoyl chloride for modification.

The affinity of antibodies before and after modification was determined by indirect immunoassay using peroxidase-labelled antispecies antibodies.

2.3. *Virus reproduction*

A monolayer of permissive MDCK cells was infected with influenza A/Chili virus at 10 PFU per cell. 3.5 h post-infection the antibodies were added to the cell culture at a concentration equal to their hemagglutination titre (about 10 μg/ml). 8.5 h post-infection the cells were thoroughly washed twice with 2 vols of the medium to remove the antibodies, then incubated for 1 h with 2 vols of the medium, and washed once again with 5 vols of the same medium.

The cells were incubated for 24 h with fresh (devoid of antibodies) culture medium. Then the culture medium was separated and lightened by double centrifugation at 6000 × g for 15 min. The infection (1 g PFU/ml) and hemagglutination (HAU/ml) titres were assayed in the supernatant as detailed in [10].

3. RESULTS AND DISCUSSION

To introduce stearic acid residues into antibody molecules, the latter were modified with stearoyl chloride using the system of surfactant reversed micelles in an organic solvent (Aerosol OT in octane) [5,9]. The modified antibodies contained two residues of stearic acid per protein globule and retained 80% activity in comparison with initial (non-modified) antibodies.

The data presented in fig.1 indicate that incubation of influenza A/Chili virus-infected MDCK cells with non-modified polyclonal antibodies against this virus for several hours does not practically affect the development of infection. Under these conditions hydrophobized antiviral antibodies decrease the virus reproduction almost by 2 orders.

Special experiments have proved that hydrophobized antibodies do not produce a toxic effect on infected cells, i.e., a decrease in virus reproduction observed is not connected with cell destruction in the presence of antibodies.

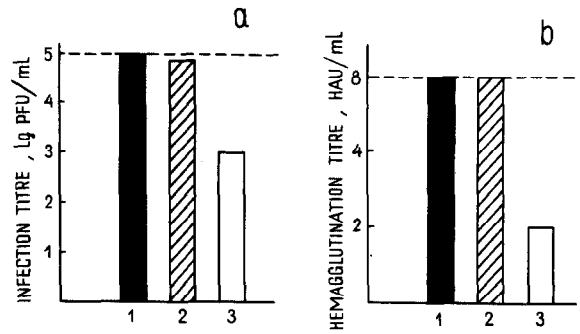

Fig.1. Reproduction of influenza A/Chili/1/83(H1N1) virus in permissive MDCK cells in the presence of: (1) stearoylated normal mouse IgG; (2) non-modified; and (3) stearoylated polyclonal antibodies against influenza A/Chili virus. (a) Infection titre. (b) Hemagglutination titre. The dotted lines show the values of infection and hemagglutination titres in the control experiment (in the absence of antibodies).

It should be noted that after acquiring an ability to suppress virus reproduction in cells, hydrophobized antibodies retain their specific properties: the addition of stearoylated normal IgG, which are not able to interact specifically with influenza virus antigenic determinants, does not affect virus reproduction in infected cells (fig.1).

The data obtained do not fully account for the mechanism of the discovered phenomenon. However, it is highly probable that hydrophobized antibodies penetrate into infected cells and block the assemblage of virus particles and/or the synthesis of virus components. Stearoylated monoclonal antibodies against the NP-protein (the internal antigen of the influenza virus, which is accessible to antibodies only inside the cell [11]) possess a considerable antiviral activity (data not presented). These findings certainly support the above made assumption.

In any case, we believe that the method of suppression of virus reproduction by artificially hydrophobized antiviral antibodies can be applied for the therapy of different viral infections, and, in particular, those that have no safe methods of treatment yet. In this connection the key problem is whether artificially hydrophobized antiviral antibodies can penetrate into infected cells and ac-

cumulate there suppressing the development of infection. In other words, can antibodies be directly transported to antigens, separated from them by cellular membranes? Recently, a principle of imparting transmembrane properties to proteins [5] has been successfully applied for the directed transport of antibodies across the hemato-encephalic barrier in the brain [12]. These findings certainly give ground to a positive answer to this question.

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