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A study of the NS3 nonstructural protein of tick-borne encephalitis virus using monoclonal antibodies against the virus

A. V. Timofeev[1], Alla A. Kushch[2], M. F. Vorovitch[1], S. M. Tugizov[2], L. B. Elbert[1], and D. K. Lvov[2]

[1] Institute of Poliomyelitis and Viral Encephalitides, Moscow
[2] D.I. Ivanovsky Institute of Virology, Moscow, U.S.S.R.

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Summary. We obtained two monoclonal antibodies against native NS3 nonstructural protein of tick-borne encephalitis (TBE) virus, strain Sofyin. Using these monoclonal antibodies, we were able to determine that NS3 is a stable protein with preferential perinuclear localization. Both monoclonal antibodies precipitate NS3 proteins of TBE strains Sofyin and Absettarov and of Langat virus but do not precipitate analogous proteins of other flaviviruses (Powassan, yellow fever, Japanese encephalitis). Taking into account the work of others, we suggest that there are at least two distinct epitopes on NS3 protein. A putative functional model of NS3 is discussed.

Introduction

The NS3 protein is one of the major virus-specific components of cells infected with flaviviruses. This protein can be detected early in infection, the maximum rate of synthesis greatly depending on the multiplicity of infection [9]. By comparing genetic maps of flavivirus NS3 proteins we conclude that: (1) NS3 is one of the most conserved virus-specific proteins and is highly hydrophilic; the variable domain is only on the C-terminus and (2) in most flaviviruses NS3 has two conserved cysteines, in positions 1666 and 2068, and except for NS3 of tick-borne encephalitis (TBE) virus, has no potential sites for glycosylation [2, 6, 13; A.G. Pletnev, pers. comm.].

The cleavage site at the N-terminus of NS3 includes a pair of basic amino acids (Lys–Arg or Arg–Arg) in positions −2 and −1 relative to the point of cleavage, followed by an amino acid with a short side chain (Gly or Ser). Although no cellular proteases with such specificity have been found, the

existence of a virus-specific protease has been predicted [10]. The analogous protease may act on the C-terminus of NS3. Processing of the NS3 protein takes place in the cytoplasm [2]. Until now, the function of NS3 has not been clear. Recently, two groups independently predicted the dual nature of this protein: as a helicase and as a virus-specific protease [1, 3, 4].

One of the main tools for studying the structural and functional features of proteins is monoclonal antibodies (Mabs). We therefore prepared two Mabs against native NS3 nonstructural protein of TBE virus and studied their type-specificity and certain features of the NS3 protein. The results of these studies and a hypothesis of the function of NS3 are presented in this paper.

Materials and methods

Cells and viruses

Porcine embryo kidney (PE) cells were propagated in Medium 199 supplemented with 10% bovine serum. Flaviviruses used were TBE virus (strains Sofyin and Absettarov), Langat, Powassan, Japanese encephalitis, and yellow fever (17D vaccine strain).

Preparation of radioactive cell lysates

Monolayers of PE cells were infected with 0.1–1.0% clarified suspensions of brains of flavivirus-infected suckling mice at an MOI of about 1–5 plaque-forming units/cell. At 20 h after infection, the medium was replaced with Earle's balanced salt solution supplemented with 1% newborn bovine serum containing ^{14}C-amino acid at 30 μCi/ml. At the end of the desired labeling period (20 h for long labeling experiments and 1 h for pulse-labeling) the medium containing radioactive amino acids was replaced either with Medium 199 containing 0.25% newborn bovine serum for different time intervals (chase experiments) or with radioimmunoprecipitation assay buffer (0.13 M NaCl, 10 mM Tris HCl, pH 8.0; 1 mM EDTA; 1% Nonidet P-40; 500 units/ml aprotonin). After homogenization and clarification, the sample was stored at −70 °C.

Cell fractionation procedure

After radioactive labeling, PE cells were collected and fractionated as described by Grun and Brinton [6]. Briefly, after homogenization the disrupted cells were pelleted by centrifugation at 1,000 × **g** for 5 min. The supernatant fluid was separated by high-speed centrifugation (105,000 × **g** for 1.5 h) into cytoplasmic and plasma membrane fractions. After additional homogenization and centrifugation (10,000 × **g** for 10 min), the pellet from the first centrifugation was separated from nuclei and large cell debris. After this procedure, the supernatant contained fragmented outer nuclear-associated membranes. Each fraction was equilibrated by volume and stored at −70 °C.

Preparation of monoclonal antibodies

Balb/c mice were intraperitoneally inoculated five times at weekly intervals with 0.2 ml volumes of a 10% clarified suspension of brains from suckling mice infected with TBE virus (strain Sofyin). For the first three immunizations antigen was inactivated with formalin and mixed with complete Freund's adjuvant. The last two immunizations were with live virus-