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EFFECT OF THE MULTIPLICITY OF INFECTION ON SYNTHESIS OF THE TICK-BORNE ENCEPHALITIS VIRUS-SPECIFIED PROTEINS DURING A SINGLE REPRODUCTION CYCLE

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Summary. — The rate of the synthesis of tick-borne encephalitis (TBE) virus-specified proteins at high multiplicity of infection (MOI of 100 PFU per cell) was the highest by 8—14 hr post-infection (p.i.) as compared to the lower MOI of 4 PFU per cell (14 to 17 hr p.i.). The first virus-specific proteins appeared in cells from 2 to 5 hr p.i.

Key words: flavivirus; multiplicity of infection; virus-specific proteins; reproduction cycle

Introduction

The following virus-specific proteins are synthesized in flavivirus-infected cells: p98 (NV5), p71 (NV4), p51 (V3, E), p44 (NV3), p21 (NV2^1/2), p19 (NV2), p14 (NV 1 1/2), an analogue ofvirion protein V2 (C) and p10 (NV1) (Westaway *et al.*, 1980). In addition to proteins V3 and V2, the protein p8 (V1, M) has also been detected in virions. In TBE virus-infected cells, the synthesis of an additional virus-specific protein p79 (NV4^1/2) has been observed (Svitkin *et al.*, 1978; Lyapustin *et al.*, 1980).

The synthesis of flavivirus-specific proteins was studied at late post infection intervals from 24 to 48 hr p.i. in cell culture (Shapiro *et al.*, 1971; Trent and Quereshi, 1971; Westaway, 1973; Westaway *et al.*, 1977; Heinz and Kunz, 1982), i.e. for longer than a single reproduction cycle which lasts up to 25 hr. The present paper deals with the analysis of proteins synthesized in TBE virus-infected cells during a single reproduction cycle, i.e. since the moment of adsorption to the release of the majority of infectious virions from infected cells.

Materials and Methods

TBE virus (strain Sofyin) was passaged in newborn albino mice. The reproduction of continuous cell culture of swine embryo kidney (SEK) and its infection with virus-containing suspension of the mouse brain was described earlier (Lyapustin *et al.*, 1983).

TBE virus-specific proteins were labelled with a mixture of ^{14}C-amino acids (740 MBq — 1.48 GBq/ml, Amersham) added to the maintenance medium. Before introduction of radioactive

amino acids, the cells were pretreated with 5 μg/ml of Actinomycin D (Lyapustin *et al.*, 1980). After the labelling had been finished, the maintenance medium was removed, the cells were washed, lysed and analysed in 10 and 15 % polyacrylamide gel using a discontinuous buffer system (Laemmli, 1970). Gel fluorography has been conducted as described by Bonner and Laskey (1974). Virion proteins were prepared as described earlier (Lyapustin *et al.*, 1980).

Infectivity of TBE virus was estimated by means of plaque method in SEK cell culture (Dzhivanyan *et al.*, 1974).

Results

Fig. 1 shows the electrophoregrams of proteins synthesized in TBE virus-infected SEK cells at different intervals (from 8 to 23 hr p.i.) and at different MOI (4 PFU and 100 PFU per cell, respectively). The following virus-specific proteins were clearly seen in 15% polyacrylamide gel: p93 (NV5), p79 (NV4^1/2), p69 (NV4). p53 (V3, E), p21 (NV2^1/2), p18 (NV2), p13 (NV1^1/2) and p12 (NV1). During the analysis of cell lysates in 10% polyacrylamide gel the protein p81 indistinguishable from protein p79 in 15% polyacrylamide gel was detected (Fig. 2). In infected cells, the virus-specific proteins p24 and p15 were also found. Protein p24 could be differentiated from a cellular protein (which has a somewhat higher mobility) in cell lysates infected at high MOI (100 PFU per cell). At a lower MOI this cellular protein was synthesized even more actively, thus masking the presence of virus-specific p24 protein.

The intensity of the synthesis of virus-specific proteins during a single virus reproduction cycle depended on the infectious dose. At high MOI of 100 PFU per cell the synthesis of virus-specific proteins culminated from 8 to 14 hr p.i. Later on, the rate of protein synthesis was significantly decreased (Fig. 1, lanes 2—6). At lower MOI of 20 PFU per cell, the synthesis of virus-specific proteins exhibited the same pattern, but the intensity of the protein synthesis was somewhat lower after inoculation of 100 PFU per cell, the period of active synthesis of virus-specific proteins lasting up to hr 17 p.i. At lower MOI of 4 PFU per cell, small amounts of virus-specific proteins were synthesized from 8 to 11 hr p.i., but their synthesis culminated from 14 to 17 hr p.i. (Fig. 1, lane 9). Later on the intensity of synthesis of the proteins p83, p79, p69, and p53 increased while that of proteins p13 and p12 remained unchanged and that of proteins p21, p18, and p15 decreased.

It should be noted that the decrease of intensity of virus-specific protein synthesis observed from 17 to 23 hr p.i. at high MOI was also associated with a higher inhibition of cellular protein synthesis than during active synthesis of virus-specific proteins, i.e. from 8 to 17 hr p.i. (Figs 1, 2).

When following the proteosynthesis in TBE virus-infected SEK cells at intervals earlier then 8 to 11 hr p.i., the protein p69 was first detected (2—5 hr p.i.) in small quantities (Fig. 2). During the next 3 hr, proteins p93, p69 and p53 were synthesized in noticeable amount. In the course of prolonged electrophoresis in 10% gel, the protein band p53 became divided but the position of each of the two bands coincided with the position of the broad band of p53 virion protein (V3, E) in the gel. It is most likely that the protein p53 is produced in 2 forms which do not differ in the length of their

polypeptide chain but in the extent of glycosylation, which is responsible for their different electrophoretic mobility. Proteins p81 and p79 were synthesized in low quantities from 5 to 8 hr p.i., later on their synthesis was better expressed. During the analysis of cell lysates in 15% gel no synthesis of low molecular virus-specific proteins was revealed at 3—5 hr and at 5 to 8 hr p.i., respectively.

Discussion

The set of high-molecular virus-specific proteins of TBE virus found in SEK cells is similar with respect to quantity and molecular mass to the set of proteins found in the chick fibroblast cells (Heinz and Kunz, 1982). Protein p47 (NV3) is well seen in chick fibroblast cells, whereas in SEK cells it can be detected only after treatment of cells with hypertonic NaCl solution (Lyapustin *et al.*, 1980). Low-molecular proteins differ significantly. In chick fibroblasts infected with TBE virus, the synthesis of virus-specific proteins p24—25, p15 (V2, C), and p14.5 (NV1^1/2) was observed, but the proteins p21, p18, and p12 synthesized in infected SEK cells together with p24 and p13 (V2, C) were not detected (Fig. 1). Probably, the production of low-molecular mass virus-specific proteins is different in mammalian cells (SEK) as compared to avian ones (chick fibroblasts). These differences may be due to the action of cellular proteases of different specificities participating in the cleavage of supposed precursors of the low-molecular virus-specific flavivirus proteins.

Basic characteristics of single reproduction cycle have been studied for flaviviruses Kunjin, West Nile, dengue-2, Saint-Louis encephalitis, Japanese encephalitis and Uganda S, both in the cell cultures of vertebrates (Stollar *et al.*, 1967; Trent *et al.*, 1969; Shapiro *et al.*, 1971; Fukui, 1973; Westaway, 1973; Wengler *et al.*, 1978) and in mosquitoe cell cultures (Wengler *et al.*, 1978; Ng and Westaway, 1979). Latent period of infection was about 13 hr and the time of release of the bulk of infectious viral particles was 23 to 27 hr. The production of infectious TBE virus particles in vertebrate cells (Andzhaparidze and Bogomolova, 1962; Avakyan *et al.*, 1963; Heinz and Kunz, 1977), as well as in the body of natural tick carrier (Chunikhin and Kurenkov, 1980) meets the same regularities as of the mosquito-borne flaviviruses. Synthesis of virus-specific RNAs of flaviviruses, TBE virus including, is most active during the period from 12 to 20 hr p.i. (Stollar *et al.*, 1967; Trent *et al.*, 1969; Soloviev *et al.*, 1971; Fukui, 1973; Boulton and Westaway, 1977). Data on synthesis of virus-specific proteins at early intervals of reproduction cycle, to our knowledge, are not available in literature, thus the presented work was the first research dealing with this problem.

Within the first 5 hr p.i. no noticeable synthesis of virus-specific proteins was observed except for low synthesis of the protein p69. Furthermore, noticeable amounts of proteins p93, p69, and p53 were synthesized. At high MOI these and other virus-specific proteins were most efficiently synthesized within 8 to 14 hr p.i., i.e. at the end of latent period and during the release of the first newly formed infectious particles from the cells. Later on, i.e. during active release of infectious particles from the cell, the synthesis of

virus-specific proteins was decreased. In the course of lower MOI of 4 PFU per cell, the synthesis of virus-specific proteins was essentially different. The period of their highest synthesis was shifted to 14—17 hr p.i., the rate of synthesis of individual proteins varying at different intervals.

The data of Trent *et al.* (1969) are important for interpretation of the differences in the synthesis of virus-specific proteins of TBE virus depending on the infectious dose. These authors have shown that the quantity of cells primarily infected with Saint-Louis encephalitis virus is about 90% at any multiplicity ranging from 5 to 90 PFU per cell. The method of infective centres used by these authors did not allow them to estimate the number of infectious particles entering a separate cell at different infectious doses or the extent of development of pathological processes in the cell as related to the infectious dose, whereas electrophoretic analysis of the proteins allows to register the dose-dependent differences in the course of infection.

It is most likely that at high MOI a large quantity of viral particles enter the cell, and after genomic RNA is released from them, virus-specific proteins are synthesized. Upon infection at a MOI of 4 PFU per cell, several viral particles seem to penetrate into some cells, and the low synthesis of virus--specific proteins observed at earlier times (8—11 hr) is caused by translation of proteins with RNAs released from virion particles, i.e. the proteins of these cells are produced in the same way as in cells infected at 100 PFU per cell. At MOI of 4 PFU per cell the majority of cells are likely to be penetrated by 1 infectious virus particle, the formation of virus-specific proteins in these cells takes the time needed for the synthesis of adequate amounts of viral RNA copies required for an efficient translation. The studies of the kinetics of the release of infective particles from the cells infected at different MOI can hardly provide experimental evidence for our suggestions, because of reinfection of cells by viral particles released from some cells after the latent period, as well as because of difficulties involved with washing residual virus from cells after infection using high doses. It cannot be excluded that comparative study of the qualitative composition of virus-specific RNAs of flaviviruses synthesized in the cells within a single reproduction cycle would provide an explanation of the cause of differences in the synthesis of virus-specific proteins in the cells infected at high and low MOI, respectively.

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Explanation to Figures (Plates XXVIII—XXIX):

Fig. 1. Electrophoretic analysis of the proteins synthesized in uninfected SEK cells (lane 1) and those infected with TBE virus (lanes 2—10) in 15 % PAG. The cells were infected at a MOI of 100 PFU per cell (lanes 2—6) and of 4 PFU per cell (lanes 7—11), respectively; the label was introduced at indicated intervals p.i.
Here and in Fig. 2 the digits above indicate intervals p.i. in hr; the lanes are marked below and the designation of protein band is on either side.

Fig. 2. Electrophoretic analysis of the proteins synthesized in uninfected SEK cells (lane 8) and those infected with TBE virus (lanes 1—7) in 10 % PAG. SEK cells were infected at a MOI of 100 PFU per cell. Lane 9 shows purified virion proteins.