Topography and Dynamics of Synthesis of Structural Proteins of Newcastle Disease Virus

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Received for publication 18 December 1965

ABSTRACT

ZHDANOV, VICTOR M. (The D. I. Ivanovsky Institute of Virology, Moscow, USSR), NONNA B. AZADOVA, AND LEONID V. URYVAYEV. Topography and dynamics of synthesis of structural proteins of Newcastle disease virus. *J. Bacteriol.* **91**:1902-1906, 1966.—Newcastle disease virus S and V antigens are synthesized in the cytoplasm, as revealed by the immunofluorescence method. In some experiments, S antigen was found also in the nucleoli. Actinomycin D moderately decreased the titer of infectious virus and V antigen and accelerated the time of appearance of mature virus. Proflavine sharply decreased the synthesis of both antigens and the release of mature virus.

Previous studies of Newcastle disease virus (NDV) have shown that both protein components, the S and V antigens, are synthesized in the cytoplasm (4). Whether the virus undergoes a complete or an abortive cycle of multiplication depends upon the nature of the host cell. In the paper cited the sites of virus protein synthesis were examined by immunofluorescence, employing S and V antisera obtained by the method of Lief and Henle (3).

This method, however, does not exclude cross-reactions between S and V components. Therefore, we considered it worthwhile to study the topography and dynamics of the synthesis of structural proteins of NDV by use of highly purified S and V antisera. In addition, the influence of actinomycin D and proflavine on virus synthesis was investigated. Actinomycin D inhibits the synthesis of deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA); proflavine reacts with both DNA and RNA (6). It was hoped that study of the reproduction of NDV under these conditions would shed new light on the synthesis of structural proteins and the formation of the mature virus.

MATERIALS AND METHODS

Virus. The T strain of NDV was obtained from the Moscow Veterinary Institute. Its infectivity titer in chick embryos was 10^8 ID₅₀, and its hemagglutination titer was 1:640 with 1% chicken erythrocytes.

Tissue culture. Primary trypsinized chick embryo fibroblasts, HEp-2, and HeLa cells were grown in medium 199.

For synchronization of the infectious process, the

cultures were infected with 100 ID₅₀ of the virus per cell. Liquid fractions of virus-infected cultures and cell fractions, removed by ethylenediaminetetraacetic acid, were collected for virus titration at various intervals after infection. Accumulation of the virus and its components was determined in the cellular and liquid fractions of the culture by means of infectivity titration in chick embryos, hemagglutination reaction, and complement-fixation test with S antiserum.

Immunofluorescence. Purified S and V sera were obtained by a method elaborated by us (7). The conjugation of the serum with fluorescein isothiocyanate and the treatment of the preparation by an indirect method were described elsewhere (10).

Actinomycin D was supplied by Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., to whom we express our sincere gratitude. The cell cultures were treated with 0.5 µg/ml of actinomycin D 2 hr prior to infection.

Proflavine (5 µg/ml) was introduced with medium 199 30 min after infection.

RESULTS

The dynamics of the reproduction of the virus were similar in the three cell systems studied, with the exception that the titers of all components were higher in chick fibroblasts than in HEp-2 or HeLa cells. Therefore, only data obtained with HEp-2 cells are given (Fig. 1 and 2). S and V antigens and infectious virus appeared in the cellular fraction of the culture 6 hr after infection, and reached a maximum after 12 to 13 hr. The virus and its components appeared in the tissue culture fluid 2 to 3 hr later.

In the immunofluorescence experiments, the first traces of S antigen were observed in the peri-

FIG. 1. Dynamics of synthesis of components of NDV in cellular fraction (HEp-2). (A) Complement-fixing (S) antigen; (B) hemagglutinin; (C) infectious virus; ●, controls; ■, with proflavine; *, with actinomycin D.

FIG. 2. Appearance of components of NDV in tissue culture fluid (HEp-2). Designations as in Fig. 1.

nuclear zone of the cytoplasm 6 hr after infection. As infection proceeded, fluorescence spread to the periphery of the cytoplasm (8 to 10 hr), and filled the cytoplasm within 12 to 15 hr (Fig. 3). In several cases a progressive increase in fluorescence of the nucleoli was observed at later periods of infection (Fig. 4).

V antigen appeared in the cytoplasm at the same time as S antigen, but its fluorescence was more diffuse and was often observed at the periphery of the cytoplasm (Fig. 5). Neither the nuclei nor the nucleoli fluoresced during the period of observation.

Action of actinomycin D. The treatment of the cells with actinomycin D caused a moderate decrease in the titer of infectious virus and hemagglutinins, with no decrease in S antigen. A slight acceleration in the time of appearance of infectious virus was observed both in the cellular and extracellular fractions of the culture (Fig. 1 and 2). In immunofluorescence experiments, no difference was noted between actinomycin-treated and untreated cultures (Fig. 6).

Action of proflavine. In cell cultures treated with proflavine, a marked decrease in synthesis of structural proteins and in release of mature virus was observed. The synthesis of hemagglutinin was much more inhibited than was the synthesis of S antigen (Fig. 1 and 2).

Cells treated with proflavine manifested symptoms of degeneration with loss of cytoplasm, which remained as a narrow rim around the nucleus. Both S and V antigens were seen as granular accumulations which only partially filled the cytoplasm. Fluorescence of the cytoplasm was less intense throughout the period of observation than in cells not treated with proflavine (Fig. 7).

DISCUSSION

A comparison of the data on the synthesis of S and V antigens and the formation of mature virus with the data obtained by the immunofluorescence method suggests that the reproduction of NDV, beginning with the synthesis of the structural proteins and concluding with the formation of mature virus, takes place in the cytoplasm. The ap-

FIG. 3. Synthesis of *S* antigen of NDV in HEp-2 cells as revealed by the indirect immunofluorescent method with *S* antibody. Hours after infection: a, 6; b, 8; c, 10; d, 12; e, 15.

FIG. 4. Accumulation of *S* antigen of NDV in the nucleoli of HEp-2 cells. Hours after infection: a, 6; b, 9; c, 12.

FIG. 5. Synthesis of *V* antigen of NDV in HEp-2 cells. Hours after infection: a, 6; b, 8; c, 10; d, 12; e, 15.

FIG. 6. Action of actinomycin D ($0.5 \mu\text{g/ml}$) on synthesis of S and V antigens in HEp-2-cells. Hours after infection: a (S antigen), 10; b (V antigen), 10.

FIG. 7. Action of proflavine ($5 \mu\text{g/ml}$) on synthesis of S and V antigens of NDV in HEp-2 cells. Hours after infection: a (S antigen), 8; b (S antigen), 12; c (V antigen), 10.

pearance of S antigen in the nucleoli at the late stages of infection might be due to the transportation of it from the cytoplasm. According to Wheelock (8), the synthesis of RNA probably takes place here also, although the autoradiographic data presented do not exclude the possibility that the RNA is synthesized in the nucleus and rapidly transported into the cytoplasm.

Our experiments with actinomycin D seem to support the first assumption. The binding of actinomycin D to nuclear DNA structures should influence the synthesis of viral RNA were it taking place in the nucleus in a manner analogous to that observed in the case of fowl plague virus (5). The absence of such a steric effect of actinomycin D on NDV (9) lends support to the assumption that all components of this virus are synthesized in the cytoplasm.

Therefore, the reason that proflavine caused a more intensive inhibition of NDV than of other myxoviruses, whose components are synthesized in the nucleus as well as in the cytoplasm, is evident. In experiments with Sendai virus, for example, proflavine presumably altered the transport of S antigen from the nucleolus to the cytoplasm (1), without essentially altering the dynamics of synthesis in the nucleus and nucleolar structures. Synthesis in the latter is affected by proflavine later than in cytoplasmic structures.

Proflavine inhibits both synthesis and transport of structural proteins of NDV. Probably, it is damage to the system of intracellular transport in cells treated with proflavine which prevents the appearance of S antigens in nucleoli. This phenomenon does not occur in cells treated with acti-

nomycin D, probably because nucleolar structures themselves are damaged by this antibiotic (2).

In spite of the fact that both structural proteins of NDV are synthesized in the cytoplasm, damage to the system of intracellular transport presumed to be caused by proflavine would interfere with formation of viria and the synthesis of infectious virus. Alteration of nuclear structures by actinomycin D, however, does not interfere with synthesis of structural proteins and formation of mature NDV.

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