Acta virol. **13** : 257—263, 1969

ELECTRON MICROSCOPIC STUDY OF CYTOPLASMIC INCLUSIONS IN CELLS INFECTED WITH MEASLES VIRUS

E. ANISIMOVÁ, I. MAREŠ, F. KYNCL

Research Institute of Immunology, Prague, Czechoslovakia

Received February 10, 1969

Summary. — Cytoplasmic inclusions in BSC-1 cells infected with virulent measles virus (Edmonston strain) were studied by electron microscopy. Development of the inclusions in the cytoplasm was preceded by the appearance of filamentous structures 250 to 340 A in diameter, distributed diffusely mainly in the perinuclear zone. As the infection progressed, the filamentous structures became longer and more numerous. The first inclusions had the character of aggregates composed of sporadically and randomly distributed and partly oriented filamentous structures of tubular appearance, 250—340 A in diameter, with an axial canal and indented surface. Later, condensation of the aggregates took place and denser inclusions developed. They were composed of filamentous structures 140—160 A in diameter partly oriented later on and superficially resembling the internal component of measles virus. Inside and near the inclusions many particles, closely resembling the measles virus in morphology and size, were found.

Introduction

Cultivation of the measles virus in tissue culture (Enders *et al.*, 1954) has opened wide possibilities of studying this virus by various methods.

Electron microscopic studies on the intracellular development of the measles virus have revealed cytoplasmic and frequently intranuclear inclusions in the infected cells. Nearly all investigators have come to the conclusion that the inclusions represent not aggregates of virus particles (Black *et al.*, 1959; Kallman *et al.*, 1959; Baker *et al.*, 1960) but accumulations of randomly and sporadically distributed filamentous structures in the cytoplasm. In the nuclei, similar structures showed regular arrangement in most cases, up to the development of crystal-like masses. These observations have been confirmed and extended by other authors (Tawara *et al.*, 1961; Nishi *et al.*, 1962; Tawara, 1965).

The present paper illustrates an approach to the study into a virus-cell interaction characterized by several distinct features. An attempt was made to depict the dynamics of the process of infection.

Materials and Methods

The measles virus (Edmonston strain), the BSC-1 cell cultures and the mode of infection were the same as described previously (Anisimová *et al.*, 1968).

To study the early stages of the infection process, starting with adsorption of virus particles on the surface of the cells and ending with their penetration into the cells, the method recommended by Marcus (1959) was used: the cell suspension was infected with fresh virus portions three times in succession. Adsorption was performed at 0—2° C for 4 hours. The cell-virus complex was immediately fixed at 0° C, or was stored at 37° C for 5, 10 and 15 minutes and fixed afterwards.

Electron microscopic investigations were carried out 30 and 60 minutes after infection and then at daily intervals until the 8th day. Control cell culture specimens were examined at the same intervals. The preparation of specimens was the same as described previously (Anisimová *et al.*, 1968).

Results

The first signs of cytopathic changes in the infected cells were revealed on the 2nd day after infection; on the 3rd and 4th day the cytopathic effect involved about 30 and 70% of the cells, respectively; it reached the maximum on the 5th day. On the 7th and 8th day the major portion of the monolayer degenerated and separated from the glass. The remaining portion represented non-fused mononucleate or giant cells with a few nuclei in which signs of degeneration were only slightly expressed. This apparently was a sign of the development of the next cycle of infection.

As shown by electron microscopy of the infected cells, a specific sign of the infection was the occurrence of filamentous structures in the cytoplasm of giant cells, symplasts, and individual cells. The distribution of the filamentous structures varied at various stages of the infectious process.

Two days after infection, a small number of filamentous structures were distributed diffusely and without certain orientation mostly near the nucleus and less frequently in the peripheral areas of the cytoplasm. Their maximal length did not exceed 500 nm. In some of the cells the filamentous structures were already more numerous and their length amounted to 1000 nm (Fig. 1). In some cases the filamentous structures were localized inside the vacuoles of various size. This seems to be the result of the defence reaction of the cell (Fig. 2).

Three days after infection, whole aggregates of various form and size prevailed in the infected cells. The aggregates were composed of filamentous structures either randomly distributed or showing partial orientation, when they were located more or less parallely to each other (Figs 3 and 4). In most cases, the aggregates were localized in the perinuclear zone. In such cases, the filamentous structures were distributed on one or two sides of the nucleus or, sometimes, they surrounded the whole nucleus.

As to their ultrastructure, high-power magnification showed that the filaments most probably represent tubular structures with an axial canal 100—140 A in width and an indented surface owing to which it was most difficult to determine precisely their outer diameter ranging between 250 and 340 A (Fig. 5).

In addition to the aggregates described above, in which filamentous structures were mainly loosely distributed, denser inclusions with filamentous

structures packed closely one to another were mostly found 3 and 4 days after infection (Fig. 6). Due to high electron density of these inclusions, the filamentous structures were generally less distinct than in the previous cases. Such compact inclusions were sometimes found inside cavities, there being a fairly wide free space around the inclusions which, at low magnifications, appeared as a light halo (Fig. 7). Inclusions of this type were mostly composed of thin filamentous structures 140—160 A in diameter. However, inclusions with simultaneously occurring two types of filamentous structures, 250 to 340 A and 140—160 A in diameter, were also found. This seems to suggest a differentiation, in consequence of which the wide filamentous structures were replaced by the narrower ones (Fig. 8).

This type of inclusion differs from the still more compact, electron dense and sharply outlined inclusions which seem to represent the next step in inclusion development (Fig. 10). Thin filamentous structures, 140—160 A in diameter, are their basic morphological unit (Fig. 10a). In some inclusions, the filamentous structures were distributed at random, in others partial orientation was observed (Figs 9 and 10). Vesicles, myelin-like structures and altered formations adjoined some inclusions, most often from the periphery (Fig. 9). It was a characteristic feature that such compact inclusions were found either in the zone of the disintegrated cytoplasm of inside the cavities separated from the cytoplasm by a membrane (Figs 9 and 10).

We gained the impression that the cavity and the membrane were most frequently found in cells which were not heavily damaged. In cases where the cells had undergone greater destructive changes, the cavity and the membrane were not seen and the inclusion was found direct in the zone of the disintegrated cytoplasm.

In inclusions of this type we could reveal, in some cases, a membrane belonging direct to the inclusion and separating the content of the inclusion from the cytoplasm. Such a membrane in the stage of formation is shown in Figs 8 and 10: it is of a wavy and interrupted character and does not yet closely adjoin the inclusion.

In addition to those described above, we observed still another type of inclusion (4th, 5th day) which we defined as the "mature" type. A detailed description of the morphological characters of these inclusions has been presented (Anisimová *et al.*, 1968) and, therefore, we will mention them only briefly to allow a better understanding of the dynamics of the process of infection.

The "mature" inclusions, distinguished by a high electron density, were surrounded by a membrane and composed mainly of filamentous structures of tubular appearance, regular arrangement, and 140—160 A in outer diameter. In close vicinity of the inclusions and inside them we found particles either budding from the surface of the inclusion or released through the sequestration of the inclusion into individual fragments. By their morphological characters and size these particles were closely similar to measles virus. We are inclined to believe that these particles most probably represent measles virus, and the inclusions sites of accumulation of measles virus nucleoprotein (Figs 11 and 12).

In addition to the above-described new formations, peculiar to the process of infection, also certain morphological changes occurred in the cells. Thus, for instance, a considerable portion of the endoplasmic reticulum was found to be diminished in the cytoplasm. This was particularly apparent in zones of accumulated nuclei. Mitochondria underwent degenerative changes consisting mainly of the disappearance of cristae and a change of mitochondria into vacuoles. Lytic changes in the form of disintegration of the ground substance occurred in the cells at later stages of the infectious process.

Investigations on the early stage of infection (adsorption, and 10, 15, 30 and 60 minutes after infection) unfortunately failed to give the expected results. We did not succeed in obvious identification of either the adsorption of virus particles on the cell surface or their penetration into the cells. The failure was possibly due to the relatively low virus doses.

Discussion

Our observations showed that the appearance of electron-dense ("mature") inclusions, which we are inclined to regard as the site of accumulation of measles virus nucleoprotein, was preceded by the formation of diffusely distributed filamentous structures. With progressing infection, these structures increased in length and number. We do not know of any report about these early stages of development.

The next stage-aggregation of filamentous structures — has already been described by several authors (Kallman *et al.*, 1959; Mannweiler, 1965; Hsiung *et al.*, 1967). However, these authors gave no detailed description of the submicroscopic appearance and precise parameters of these structures.

As mentioned above, the filamentous structures found in the hyaloplasm matrix in the early stages of development, including the formation of aggregates, in agreement with our interpretation, have a tubular structure and their diameter equals 250—340 A. By their outer appearance, these structures resemble the well-known internal component (nucleocapsid) of the measles virus revealed by the negative staining technique and described by Waterson *et al.* (1961) and Waterson (1965). According to Waterson, the outer diameter of the internal component equals 120—150 A in a virus particle which is not much altered in this outer appearance, and 170—180 A on treatment of the virus with ether or detergent. These values are much lower than those obtained by us. On the other hand, filamentous structures 260—280 A in diameter were obtained from the cytoplasm of cells infected with the measles virus and subjected to an osmotic shock by Howatson (1962), Almeida and Howatson (1963), and Norrby and Magnusson (1965). The negative staining technique has shown that they represent a typical virus component, but slightly larger in size, of the pitch height of 70—75 A, and surrounded by an extratubular structure which seems to have a spiral configuration.

We are inclined to consider the wide filamentous structures which lie free or are gathered in aggregates to be the earliest stages of reproduction of the nucleoprotein component in the cytoplasm. The fact that these filamentous structures are disclosed mainly in the perinuclear zone of the cell and

that the "mature" inclusions, near which a number of virus-like particles are found, are localized at the same site may offer some evidence about the antigenic character of these filamentous structures. Their viral character was also confirmed by the haemadsorption experiment reported by Mannweiler (1965), in which monkey erathrocytes were fixed on the surface of cells containing filamentous-like strucures of similar outer appearance.

Further on, most probably differentiation may have taken place in the stage of development of denser inclusions, and structures 140—160 A in diameter appeared in place of the wide filamentous structures. Supporting evidence is the picture shown in Fig. 8, in which both structures are disclosed.

Another possibility, though less probable, accounting for the presence of the two types of filamentous structures, could be a different degree of susceptibility of host cells to the virus.

The next stage, which in our conception represents the next stage in inclusion development, is characterized by subsequent condensation of inclusions composed of filamentous structures 140—160 A in diameter, and by partial orientation of the latter.

As regards the development of membranes, the time of their formation probably does not coincide. At the beginning, a membrane which separates the cytoplasm from the inclusion seems to be formed and the inclusion is found laying in a smaller or larger cavity. This membrane is not formed regularly. The second membrane, which belongs to the inclusion, seems to develop later on, because we observed inclusions without their own membranes but already lying in the cavity separating them from the cytoplasm. Budding of virus-like particles could be disclosed only after a membrane own to the inclusion had been formed. We think that the light halo around the inclusions, which was seen in the optic microscope in fixed and stained preparations, represents no artifact (Black *et al.*, 1959), but either a free space which has developed as the result of separation of the cytoplasm from the inclusion, or a zone of disintegrated cytoplasm.

On comparing our data with reports on myxoviruses, we found the greatest morphological resemblance with parainfluenza virus types 2 and 3 (Reczko and Bögel, 1962; Kuhn and Harford, 1963; Compans *et al.*, 1966; Ané, 1967). Especially interesting is the finding by Tajima *et al.* (1967) of electron-dense inclusions with rinderpest virus, whose fine structure was very similar to that observed in our work with measles virus.

References

Almeida, J. D., and Howatson, A. F. (1963): A negative staining method for cell-associated virus. *J. cell. Biol.* **16**, 616—620.

Ané, C. (1967): Étude au microscope électronique de la morphogenèse d'un myxovirus parainfluenzae 3. *J. Microscopie* **6**, 31—40.

Anisimová, E., Mareš, I., and Kyncl, F.: (1968) Electron microscopic study of tissue culture cells infected with measles virus. *Acta virol.* **12**, 289—295.

Baker, R. F., Gordon, J., and Rapp, F.: (1960): Electron-dense crystallites in nuclei of human amnion cells infected with measles virus. *Nature (Lond.)* **185**, 790—791.

Black, F. L., Reissing, M., and Melnick, J. L. (1959): Measles virus *Advanc. Virus Res.* **6**, 205—227.

Compans, R. W., Holmes, K. V., Dales, S., and Choppin, P. W. (1966): An electron microscopic study of moderate and virulent virus-cell interactions of the parainfluenza virus SV 5. *Virology* **30**, 411—426.

Enders, J. F., Peebles, T. C., Chang, Y., and Holoway, A. (1954): Propagation in tissue cultures of cytopathogenic agents from patients with measles. *Proc. Soc. exp. Biol. (N.Y.)* **86**, 277—286.

Howatson, A. F. (1962): Architecture and development of tumor virus and their relation to virus in general. *Fed. Proc.* **21**, 947.

Hsiung, G. D., Bensch, K. G., and Prose, P. H. (1967): Electron microscopy of monkey kidney cells infected with both SV 40 and measles virus. *Nature (Lond.)* **215**, 178—179.

Kallman, F., Adams, J. M., Williams, R. C., and Imagawa, D. T. (1959): Fine structure of cellular inclusions in measles virus infections. *J. biophys. biochem. Cytol.* **6**, 379—382.

Kuhn, N. O., and Harford, C. G. (1963): Electron microscopic examination of cytoplasmic inclusion bodies in cells infected with parainfluenza virus type 2. *Virology* **21**, 527—530.

Mannweiler, K. (1965): Ultrastructural examinations of tissue cultures after infection with measles virus. *Arch. ges. Virusforsch.* **16**, 89—96.

Marcus, P. J. (1959): Symposium on the biology of cells modified by viruses or antigens. IV. Single cell techniques in tracing virus-host interactions. *Bact. Rev.* **23**, 232—249.

Nishi, Y., Funahashi, S., Knakawa, T., and Fukal, K. (1962): Micromorphological changes in measles infected KB cells. *Biken's J.* **5**, 45—46.

Norrby, E. C., and Magnusson, P. (1965): Some morphological characteristics of the internal component of measles virus. *Arch. ges. Virusforsch.* **17**, 443—447.

Reczko, E., and Bögel, K. (1962): Elektronenmikroskopische Untersuchungen über das Verhalten eines vom Kalb isolierten Parainfluenza-3-Virus in Kälbernierenzellkulturen. *Arch. ges. Virusforsch.* **12**, 404—420.

Tajima, M., Ushijima, T., Kishi, S., and Nakamura, J. (1967): Electron microscopy of cytoplasmic inclusion bodies in cells infected with rinderpest virus. *Virology* **31**, 92.

Tawara, J. T. (1965): Fine structure of filaments in dog kidney cell cultures infected with measles virus. *Virology* **25**, 322.

Tawara, J. T., Goodman, J. R., Imagawa, D. T., and Adams, J. M. (1961): Fine structure of cellular inclusions in experimental measles. *Virology* **14**, 410—416.

Waterson, A. P. (1965): Measles virus. *Arch. ges. Virusforsch.* **16**, 57—80.

Waterson, A. P., Cruickshank, J. G., and Laurence, G. D. (1961): The nature of measles virus. *Virology* **15**, 379—382.

Explanation of Electron Micrographs:

BSC-1 cells infected with the Edmonston strain of measles virus.

Fig. 1. Filamentous structures, 250—340 A in diameter, distributed diffusely in the perinuclear zone of the cell. Two days after infection.

Fig. 2. Filamentous structures localized inside the vacuoles of various size. Two days after infection.

Figs 3 and 4. Aggregates composed of randomly distributed and partly oriented filamentous structures 250—340 A in diameter.

Fig. 5. Cross and longitudinal sections of filamentous structures 250—340 A in diameter. Three day after infection.

Fig. 6. Inclusion in the matrix of the cytoplasm, 3 days after infection.

Fig. 7. Denser inclusion lying in a cavity. The membrane separating the cytoplasm from the cavity can be distinctly seen.

Fig. 8. Inclusion in the zone of disintegrated cytoplasm, composed of 2 types of filamentous structures: 250—340 A in diameter (centre) and 140—160 A in diameter (periphery). Onset of formation of the proper membrane around the inclusion. Three days after infection.

Fig. 9. Electron dense inclusion composed of filamentous structures 140—160 A in diameter. The inclusion lies in a large cavity separated from the cytoplasm by a membrane. Three days after infection.