MICROBIOLOGY AND IMMUNOLOGY

Infection of Highly Differentiated Human and Guinea Pig Astrocytes with Herpes Simplex and Measles Viruses

N. N. Poleshchuk, Z. B. Kvacheva, Yu. G. Ul'kevich, and E. D. Cherstvoi

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 10, pp. 413-417, October, 1996
Original article submitted June 14, 1995

The morphogenesis of type I herpes simplex virus and measles virus (Edmonston strain) is studied in primary cultures of highly differentiated human and guinea pig astrocytes. It is shown that astrocytes are involved in infection. Under varied conditions of inoculation, herpes simplex virus causes acute infection, while measles virus induces both acute and chronic infection. Chronic infection is associated with impaired assembly of virions, accumulation of thickened ribonucleoproteins (25-37 nm in diameter) in the cytoplasm, and budding of "empty" viral particles into the extracellular space. Persisting measles virus reactivates 78±9.4% of cells, which is accompanied by hyperproduction of the glial fibrillar acid protein.

Key Words: *primary cultures; human and guinea pig astrocytes; herpes simplex virus; measles virus*

Recent neuroimmunological studies show that antigen-activated astrocytes are capable of phagocytosis, production of tumor necrosis factor, interleukins-1α, -1β, -6, and α,γ-interferon, and expression of I and II class proteins of the major histocompatibility complex, which led to reconsideration of the role of astrocytes in the pathogenesis of encephalomyelitis and slow degenerative processes in the central nervous system (CNS) [4,6,8,9]. However, evaluation of the significance of astroglia in the maintenance of infectious process in the CNS and in its chronization was hampered for a long time by the absence of adequate *in vitro* models.

In the present study we compared the sensitivity of primary cultures of highly differentiated human and guinea pig astrocytes to measles virus (MV) and type I herpes simplex virus (HSV-1) with a parallel investigation of morphology, cytoarchitectonics, and expression of the glial fibrillar acid protein (GFAP) by these cells.

Byelorussian Institute of Epidemiology and Microbiology, Byelorussian Ministry of Health, Minsk; Minsk State Medical Institute

MATERIALS AND METHODS

Primary confluent cultures of human and guinea pig astrocytes were obtained as described elsewhere [5]. Astrocytes were identified by indirect immunofluorescence using anti-GFAP antiserum (Serva) and specific ultrastructural characteristics [3]. Measles virus (strain Edmonston) and HSV-1 were added to 1st-2nd passage cultures in dose ranges 0.01-0.1 and 1-10 TCD_{50}/ml during logarithmic growth. Virus accumulation was assessed by adding 10-fold serial dilutions of growth medium (from 10^{-1} to 10^{-9}) and cell lysates to test-systems: chick embryo fibroblasts for HSV-1 and L-41 for MV. The expression of viral

0007-4888/96/0010-1010$15.00

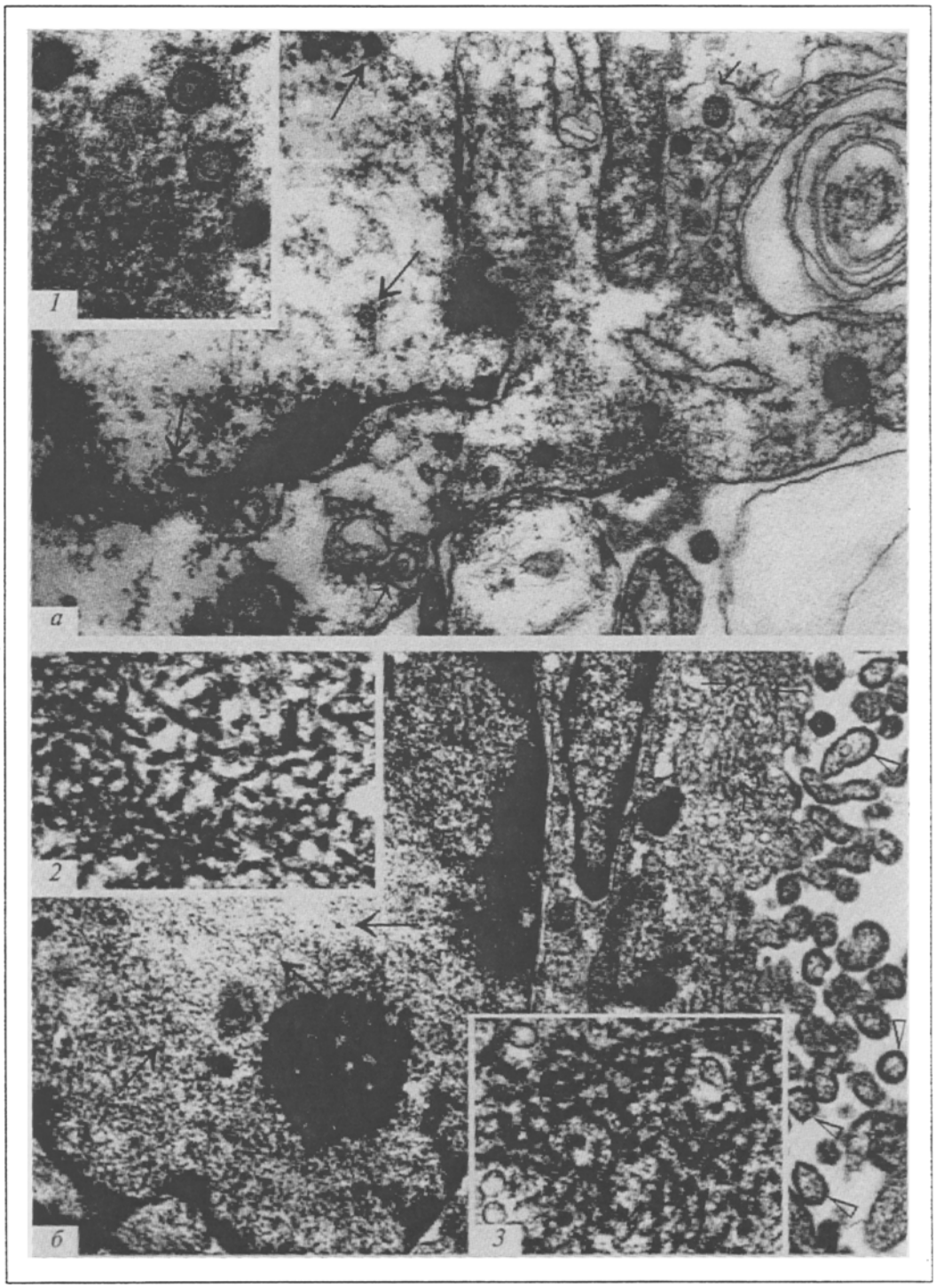

Fig. 1. Ultrastructural changes in human astrocytes infected with low doses (0.01-0.1 lg TCD_{50}/ml) of type I herpes simplex virus (day 1 after infection) and measles virus (first passage, day 14). *a*) various stages of morphogenesis of type I herpes simplex virus in the nucleus (long arrows) and cytoplasm (short arrows). *1*) nuclear capsids at a larger magnification. *b*) morphogenesis of measles virus in chronically infected astrocytes. Formation of "smooth" viral RNP 12-17 nm in diameter (*2*) in the zone of chromatin dispersion (long arrows) in the nucleus. Accumulations of "rough" RNP 25-37 nm in diameter (*3*) in the cytoplasm (short arrows). "Empty" measles virions without or with partially formed nucleocapsid (triangle arrows) in the extracellular space. Contrasting with 5% uranyl acetate and 0.4% lead citrate. *a*) ×85,200; *1*) ×212,000; *b*) ×19,100; *2*, *3*) ×48,000.

antigens in astrocytes was assessed by indirect immunofluorescence with type-specific antisera diluted 1:50 for HSV-1 and 1:20 for MV. The intensity of the fluorescence of immune complexes was expressed as —, ±, +, ++, +++, ++++.

Morphofunctional study was carried out on viable cells by phase-contrast microscopy and on fixed cells stained with hematoxylin and eosin. Morphometry was performed using a Bioskan AT+ image analyzer with specially designed software.

The state of astrocyte organelles and viral morphogenesis were assessed by electron microscopy using ultrathin sections. Preparations of culture medium were contrasted with aqueous solution of uranyl acetate (2%) and analyzed for the presence of viruses in a JEM-100CX-II electron microscope.

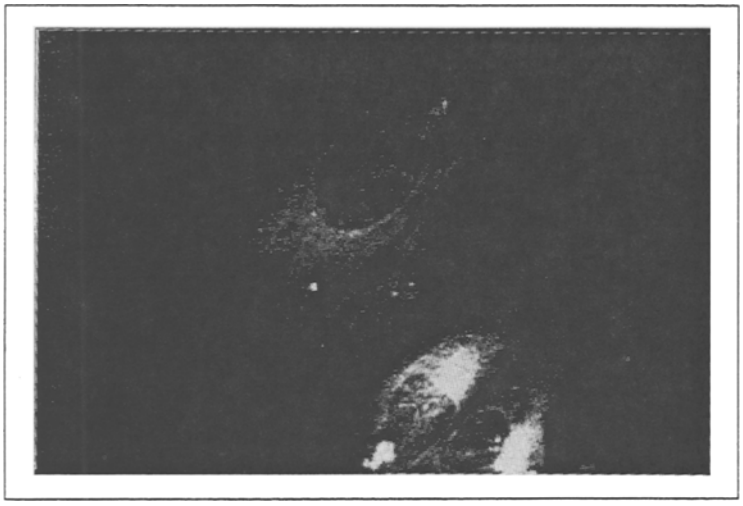

Fig. 2. Reactive human astrocytes in a culture chronically infected with measles virus (second passage). Immunofluorescent staining with antibodies against glial fibrillar acid protein. ×400.

RESULTS

High doses of HSV-1 (1 and 10 TCD_{50}) caused cytopathic changes in 10-15% of human astrocytes as soon as 1 day after infection. These changes manifested themselves as focal destruction of intermediate filaments and microtubules and formation of nuclear eosinophilic inclusions. The indirect immunofluorescence analysis revealed viral antigen in 77.5± 6.07% of cells. On day 2 after infection, the amount of cytoskeletal elements in astrocytes progressively decreased. The developing infection was characterized by the appearance of astrocytes with mitotic pathology (predominantly K metaphase) and formation of giant multinuclear cells with an area up to 35,897±1021 μ^2 (*vs.* 1923±281 μ^2 in the norm) containing 5-12 nuclei varying in shape and size. Chromatin rearrangements and swelling of nucleoli followed by their destruction were observed in multinuclear astrocytes. The titer of HSV-1 in the culture medium was 8.5 lg ID_{50}/ml. Electron microscopy revealed nucleocapsids at various stages of morphogenesis in the nucleus and cytoplasm (Fig. 1, *a*). Mature virions were seen in the extracellular space. After 3-4 days in culture, detachment of astrocytes from the substrate led to the disruption of confluence.

In low doses (0.1 and 0.01 TCD_{50}/ml), HSV-1 had a similar cytopathic effect on cultured human astrocytes. Complete disruption of the monolayer occurred on day 5-6 after infection. Reproduction of HSV-1 in astrocytes was accompanied by destruction of the majority of microtubules and almost complete degradation of intermediate filaments (Table 1). The expression of GFAP in infected cells decreased from ++++ to ++ and +.

The addition of various doses of HSV-1 to cultures of guinea pig astrocytes also led to the development of acute infection with cytolysis and destruction of cell monolayer after 4-5 days. Viral morphogenesis was similar to that in cultured human astrocytes. However, morphological changes and cytopathic effect were less pronounced and occurred 1-2 days later.

TABLE 1. Reproduction of HSV-1 and Measles Virus in Cultured Human and Guinea Pig Astrocytes

Virus/Astrocytes	Infecting dose (lg TCD_{50}/ml)	Infection	Maximum viral titer in culture medium (lg TCD_{50}/ml)	Percent of cells expressing viral antigens on day 2-4
HSV-1:				
human astrocytes	0.01-0.1	Acute	7.5	67.1±2.98
	1-10	Acute	8.5	77.5±6.07
guinea pig astrocytes	0.01-0.1	Acute	7.7	78.7±5.51
	1-10	Acute	Not determined	Not determined
Measles virus:				
human astrocytes	0.01-0.1	Chronic*	1.7-2.0	61.7±7.5
	1-10	Acute	4.7	56.5±4.46
guinea pig astrocytes	0.01-0.1	Chronic**	2.1	44.67±2.51
	1-10	Acute	3.4	53.3±7.43

Note. TCD = tissue cytopathic dose. *Three passages (110-120 days); **four passages (100-130 days).

Depending on dose, MV caused acute (1-10 TCD_{50}/ml) and chronic (0.01-0.1 TCD_{50}/ml) infection (Table 1). Cytoplasmic eosinophilic and nuclear basophilic inclusions were seen in human astrocytes on days 2-3 of acute infection. Some cells were hypertrophied (area 4280±367 μ^2) and contained 3-5 nuclei; cell fusion with formation of small clusters was also observed. In contrast to L-41, HEp-2, and other cell systems [1,2], human astrocytes did not form giant symplasts, but their shape changed from stellate to spindle-like. Virus-specific antigens were revealed by indirect immunofluorescence in 56.5±4.46% of cells 24-48 h after infection. The MV titer in the culture medium was 4.7 lg ID_{50}/ml. Focal destruction of cells was observed on day 5-7 after infection. Electron microscopy revealed occasional mature virions (80-150 nm in diameter) in the extracellular space as well as large cytoplasmic (up to 2.5 μ^2) and nuclear (up to 12 μ^2) viroplasts consisting of linear ribonucleoproteins (RNP) MV 17-20 and 14-18 nm in diameter, respectively. In some cells, the nuclei were markedly enlarged, and their membranes formed deep cristae, which created a picture of pseudomultinuclearity. At later stages of infection, changes developing in the cytoskeleton were similar to those observed in HSV-1-infected cells.

Low doses of MV induced no pronounced cytopathic changes in human astrocytes, leading to the development of chronic infection. On the second weeks of culturing, 78±9.4% astrocytes were phenotypically similar to reactive astroglia: their area increased to 6354±687 μ^2, which was accompanied by accumulation of organelles in the perikaryon and hyperproduction of GFAP (Fig. 2). In some cells, filaments formed "bundles" and "knots". After 2 weeks of growth, the cultures were passaged three times during a 110-120-day period. The MV titer remained stable throughout the observation period (1.7-2.0 lg TCD_{50}/ml). It should be noted that morphogenesis of MV in chronic infection was different: accumulations of viral RNP in the cytoplasm of reactive astrocytes looked like more compact bundles of increased diameter (25-37 nm *vs.* 15-10 nm in acute infection). Particles containing no RNP budded from the plasma membrane, while "empty" viral particles predominated in the extracellular space (Fig. 1, *b*). Only 3-5% of virions had a structure typical of mature morbilliviruses. Generally, abnormal particles budded from cells in which intermediate glial filaments were accumulated at the plasma membrane.

Different doses of MV caused acute and chronic infection in cultures of guinea pig astrocytes. Cytopathic changes (local destruction of some cells, pseudo- or true multinuclearity of 3-5% cells) were observed on day 6-8 after infection with high doses. These changes disappeared as the infection developed.

The reactivity of guinea pig astrocytes (38-47% of cells) and hyperaccumulation of GFAP (++++) were more pronounced after the addition of low doses of MV. Reactive astroglia persisted throughout the entire period of culturing. Similarly to human astrocytes, cytoplasmic and nuclear accumulations of viral RNP and "empty" virions in the extracellular space were seen. This coincided with an increase in the number of bundles with aberrant packing of gliofilaments. Such cultures persisted for 100-130 days (4 passages). The MV titer remained unchanged (Table 1).

Thus, our findings show that astrocytes are involved in HSV-1 and MV infection. Although the sensitivity of guinea pig astrocytes was lower than that of human astrocytes, they can be employed as a model for the investigation of delicate mechanisms underlying the virus/cell interaction. Astrocytes proved to be highly permissive for HSV-1 that, irrespective of infecting dose, induced degeneration of the cytoskeleton with subsequent cell lysis. Intracellular reparation was insignificant.

Morphogenesis of MV strongly depended on infecting dose. Measles virus induced both acute and chronic infection in cultures of human and guinea pig astrocytes. Reactivation of astroglia, on the one hand, and impaired assembly of virions, on the other, were observed in chronic infection. In contrast to chronically infected passage cultures of L-41, BHK, Vero, and human astrocytoma cells and primary cultures of rodent astrocytes [7,10], "empty" viral particles predominated in the extracellular space with a simultaneous intracellular accumulation of abnormally thickened viral RNP. It is most likely that intracellular rearrangements with cytoarchitectonic changes against the background of GFAP accumulation prevent the transport of RNP to the plasma membrane sites where the virions are assembled.

Using this model, we showed for the first time that human and guinea pig astrocytes can be reactivated by measles virus and maintained for an essentially long time by passaging. We believe that this model will be useful not only for the investigation of astroglial reactivation but also for the elucidation of the mechanisms and causes of immunobiological alterations of these cells. Different permissiveness of astrocytes for HSV-1 and MV may account for different rates of pathological processes occurring in CNS in acute herpetic encephalitis and relatively slow subacute sclerotizing panencephalitis caused by MV.

REFERENCES

1. O. G. Andzhaparidze, N. N. Bogomolova, Yu. S. Boriskin, *et al.*, *Vopr. Virusol.*, **33**, No. 3, 338-342 (1988).
2. O. G. Andzhaparidze, N. N. Bogomolova, I. B. Koptyaeva, *et al.*, *Ibid.*, **31**, No. 4, 430-435 (1986).
3. N. N. Poleshchuk, V. I. Votyakov, Z. B. Kvacheva, *et al.*, *Vestn. AMN SSSR*, No. 3, 18-23 (1989).
4. V. Bocchini, G. Rebel, R. Massarelli, *et al.*, *J. Dev. Neurosci.*, **6**, 525-534 (1988).
5. J. Boocher and H. Sensenbrenner, *Neurobiology*, No. 2, 97-105 (1992).
6. A. Fontana, F. Kristensen, R. Dubs, *et al.*, *J. Immunol.*, **129**, 2413-2419 (1982).
7. E. H. Macintyre and J. A. Armstrong, *Nature*, **263**, 232-234 (1976).
8. M. Pulver, S. Carrel, J. P. Mach, *et al.*, *J. Neuroimmunol.*, **14**, 123-133 (1987).
9. U. Traugott and P. Lebon, *J. Neurosci.*, **84**, 257-264 (1987).
10. S. L. Wechsler, H. C. Meissner, *Prog. Med. Virol.*, **8**, 67-95 (1982).

Cell-to-Cell Interactions in Cultures of Swine Bone Marrow Cells and CPK-66b Cells Infected with African Swine Fever Virus

N. G. Shubina, A. A. Kolontsov, M. S. Malakhova, and V. V. Makarov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 10, pp. 418-424, October, 1996
Original article submitted June 20, 1995

In the presence of specific antiserum, cultured bone marrow cells from pigs infected with African swine fever virus form cell-to-cell junctions which are morphologically similar to those observed in antibody-dependent cellular cytotoxicity. This type of cytotoxicity does not determine the reaction of delayed hemadsorption, since this reaction is realized in a long-term culture of CPK-66b cells in the absence of the effectors of antibody-dependent cellular cytotoxicity. The sensitivity of delayed hemadsorption depends on the variant of infecting virus. A negative correlation is established between the area of the contact between erythrocytes and cells infected with different variants of the virus, on the one hand, and titers of antibodies in the delayed hemadsorption reaction, on the other.

Key Words: *antibody-dependent cellular cytotoxicity; delayed hemadsorption; African swine fever virus; cell culture*

Mononuclear phagocytes are the target for the virus causing African swine fever [3,8,10]. Replicating in monocytes/macrophages, this virus induces antigenic modulations consisting in the emergence of virus-induced proteins in the plasma membrane [2,5,6]. This phenomenon manifests itself as the ability of infected leukocytes or bone marrow cells to adsorb swine erythrocytes with serotype-specific abrogation of delayed hemadsorption (DHA) [1,9]. Hemadsorption is believed to be mediated by a virus-specific protein which is functionally and structurally similar to the CD2 surface antigen of T lymphocytes [7]. These membrane-associated viral proteins are the target for immune attack involving cytolytic effector mechanisms, specifically, antibody-dependent cellular cytotoxicity (ADCC) mediated by neutrophils and specific antibodies [4]. Thus, both ADCC and DHA are characterized by the same initial stage, namely, antigenic modulation of the plasma membrane of infected cell and reaction with antibodies. Antibody binding has opposite effects on cell-to-cell interactions in a heterogeneous subpopulation of cultured swine bone marrow cells which contains infected cells: stimulation of the attachment of ADCC effector cells or inhibition of the erythrocyte ad-

Laboratory of Biochemistry, Institute of Veterinary Virology and Microbiology, Pokrov

0007-4888/96/0010-1014$15.00