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Expression of Five Viral Antigens in Cells Infected with Wild-Type and SSPE Strains of Measles Virus: Correlation with Cytopathic Effects and Productivity of Infections

By

K. P. JOHNSON[1], E. NORRBY[2], PEGGY SWOVELAND[1], and D. R. CARRIGAN[1]

[1] Department of Neurology, University of Maryland Hospital, Baltimore, Maryland, U.S.A., and

[2] Department of Virology, Karolinska Institutet, School of Medicine, Stockholm, Sweden

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Summary

Cells infected with four strains (LEC, Biken, IP-3 and DR) of subacute sclerosing panencephalitis (SSPE) virus were compared with wild type measles virus (Edmonston) with respect to titers of extracellular virus, morphology of the cytopathic effect (CPE) and occurrence of different measles virus antigens within infected cells as determined by immune fluorescence. Murine monoclonal antibodies with specifities for the nucleocapsid (NP), polymerase (P), matrix (M), hemagglutinin (H), and fusion (F) proteins as well as specific hyperimmune sera prepared in rabbits against the NP, H and M proteins were used in immune fluorescence analyses of the various strains. All of the strains produced large amounts of NP and P. Only the NP antigen occurred in nuclei of cells. The Edmonston and LEC strains also showed bright fluorescence with the antibodies against the H, F, and M antigens. Immune fluorescent intensity was variably reduced in cells infected with the Biken, IP-3, and DR strains labelled with anti M, H, or F antibodies. The Biken strain produced moderate titers of extracellular virus and moderate amounts of M, H, and F antigens whereas the DR strain produced no extracellular virus and contained no detectable M or F and only trace amounts of H antigen. The IP-3 strain was intermediate both in antigen expression and in production of extracellular virus.

Introduction

The dynamics of measles virus development *in vitro* and ultimately the pathogenesis of measles virus associated human diseases, such as subacute sclerosing panencephalitis (SSPE), may be better understood by determining the expression and intracellular location of individual viral proteins during infection. Such studies are most easily performed using monospecific antibodies directed against the

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individual viral antigens. These antibodies can be used in immune fluorescent and ultrastructural immunoperoxidase labelling studies to follow the appearance or disappearance of individual antigens and to determine their intracellular distribution. Such information may suggest how variation in the expression of viral proteins is related to changes in the viral replicative cycle seen under different conditions of infection, to the various cytopathic changes induced in cells infected with different strains of virus, and to the pathogenesis of different infections in animals or man.

We have recently been able to study the expressions of specific measles virus antigens *in vivo* (11) and *in vitro* (15) using two sets of highly defined antisera. The first set consisted of hyperimmune sera raised in rabbits against purified viral nucleoprotein (NP), matrix (M) protein, and hemagglutinin (H). These polyclonal monospecific antisera, as well as an antiserum against purified whole virus were used to study the expressions of viral antigens in an experimental model of SSPE (3, 10). It was possible to show that both NP and M antigens were produced in acutely infected hamster brains; however, as the subacute stage of disease developed, the M antigen disappeared. The virus changed from a productive to a cell-associated state et the same time. This finding is of interest in relation to the apparent absence of electrophoretically demonstrable measles M protein in brain cell cultures derived from an SSPE patient (7) and in brain extracts from three patients with SSPE (8). A defective synthesis of M protein has further been shown in studies with SSPE virus isolates in cell cultures (12, 13, 18).

The second set of antisera that have been used by us in the study of measles virus antigen expression consists of monoclonal antibodies produced in mice by hybridoma technology (15, 20). These monoclonal antibodies have specificity for the viral NP, polymerase (P), H, fusion (F) and M antigens. These antibodies were used to study cell cultures productively infected with three measles virus strains and one chronically infected cell line (15). It was shown that the temporal appearance and location of the various antigens could be determined and that differences existed in some of the virus strains tested. Of special interest was the finding that of the chronically infected cells, which all were infected, only a limited fraction produced detectable amounts of the membrane associated antigens (H, and M and in particular F).

We have now used both sets of immunologic reagents to study cell cultures infected with wild-type and four strains of SSPE measles virus including one strain (DR) which has never produced infectious virus despite numerous passages in cell culture (19). In comparison with previously employed techniques for characterization of the synthesis of virus polypeptides the immune fluorescence technique offers the advantage of allowing an evaluation of the occurrence of different virus components in individual cells. The present studies show that production of cells free virus relates to the presence of envelope antigens, in particular M antigen.

Materials and Methods

Cells and Virus

Vero cells, a continuous line of African green monkey kidney, were used in all studies and were grown as described previously (6).

The BIKEN (19) and IP-3 (2) strains of SSPE measles virus were obtained as crude 10 per cent suspensions of suckling mouse brain from Dr. P. Albrecht; Bethesda, Maryland. Their titers as plaque forming units (PFU) in Vero cells were 10^5 PFU/ml and 10^6 PFU/ml respectively. The origin of the LEC (1) strain of measles virus and procedures used for virus stock preparation have been described previously (5). Virus stock LEC-4 with a titer of 10^8 PFU/ml in Vero cells was used. The stock of Edmonston measles virus used was the supernatant of an infected Vero cell culture and had a titer of 10^6 PFU/ml in Vero cells. Vero cells chronically infected with the DR (19) strain of SSPE measles virus was obtained from Dr. H. Thormar, Staten Island, New York. The cells were stored between study periods at —70° C in culture media containing 10 percent fetal calf serum and 10 percent dimethyl sulfoxide. During passage of the cells uninfected Vero cells were added approximately once per month in order to maintain the cultures.

Production of Cell-Free Infectious Virus by the Different Virus Strains

Confluent cultures of Vero cells were infected at an input multiplicity of infection (MOI) of about one PFU/cell with the various strains of virus. When the cultures showed maximal CPE (90—100 percent involvement), the supernatant media were removed and clarified by centrifugation at $500 \times g$ for 10 minutes. For the DR strain of virus, the supernatant medium was removed from a culture showing extensive CPE (40—50 percent involvement) and was clarified as above. Serial ten-fold dilutions of these media were then inoculated onto replicate Vero cells monolayers (0.1 ml of medium dilution into four replicate cultures). These cultures were observed for fifteen days, and titers of infectious virus were calculated as Tissue Culture Infectious Dose ($TCID_{50}$) by the method of REED and MUENCH (16).

Viral Antigen Detection by Indirect Immune Fluorescence (FA)

Cells were grown in petri dishes containing sterile glass coverslips and were infected with the various strains of virus at input MOI's of 0.05—0.1 PFU/cell. Cultures of Vero cells chronically infected with the DR strain of virus were similarly grown. When CPE was widespread (50—75 per cent involvement), the coverslips were removed, rinsed in phosphate buffered saline (PBS), and fixed in cold (—20° C) acetone for 10 minutes. After air drying the coverslips were overlaid with the appropriate rabbit antisera or ascites fluid containing hybridoma antibodies for 20 minutes in a moist chamber, washed twice in PBS and incubated with either goat antirabbit 7S globulin fluorescent conjugate (Meloy Laboratories; Springlfied, Virginia) of fluorescenated $F(ab')_2$ fragment sheep antimouse IgG (Cappel Laboratories; Cochranville, Pennsylvania). Both fluorescent conjugates were used at 1:40 dilutions. The coverslips were then washed with PBS, dipped into distilled H_2O and mounted in glass slides in neutral glycerol. Uninfected Vero cell cultures served as controls. The preparations were viewed in a Leitz fluorescence microscope and appropriate fileds were photographed.

The following sera were used in the FA tests. Rabbit hyperimmune sera against measles NP, M and H antigen prepared as described previously (11, 21). Monoclonal hybridoma antibodies against 5 of the 6 measles structural components, the NP, P, M, H and F proteins, were also employed. The technique for establishment of the mouse hybridomas and the determination of the specificity of the monoclonal antibodies by radioimmune precipitation assays was presented earlier (15, 20).

Preliminary studies were performed to determine the optimum dilutions of rabbit antisera and ascites fluid hybridoma antibodies for use in the FA studies. Rabbit hyperimmune sera were diluted 1:20 to 1:80 and most ascites fluids were used in a dilution of 1:50. However, anti-NP and anti-H reagents were diluted 1:500 and 1:100, respectively. Products from two different hybridomas with specificity for each of the structural components were employed.

Fluorescence of the infected cultures was semi-quantitated using the following procedure. The most intense fluorescence seen with any virus strain and any antisera was arbitrarily designated 4+. Cultures showing no fluorescence were designed O.

Fluorescence intensities intermediate between these extremes were graded on a relative continuous scale, i.e. 3+, 2+ or 1+. Trace flurescence was designated +/—.

Hemadsorption of Infected Cultures

The procedure used has been described in detail previously (6). In summary, infected and uninfected control cultures were washed with PBS and overlaid with a 0.5 percent suspension of African green monkey erythrocytes (Flow Laboratories; Ingelwood, California) in PBS. After incubation for one hour at 37° C, the cultures were washed and observed microscopically.

Results

Viral Cytopathic Effects, Production of Infectious Cell-free Virus and Hemadsorption of Infected Cultures

The CPE produced by Edmonston, Biken, and IP-3 virus strains were similar and consisted of large, round spreading syncytia with nuclei that collected centrally. Syncytia produced by all three strains spread and resulted in an eventual involvement of the entire cell sheet. In contrast, the DR virus strain produced small angular syncytia composed of only a few nuclei which degenerated rapidly without capacity to spread. The LEC strain of virus produced a mixture of syncytia, similar to those of the Edmonston, Biken, and IP-3 strains, and rounded cells, a CPE that has been described previously (4).

Table 1. *Measles virus production, hemadsorption, and identification of specific viral antigens using specific rabbit antibodies to NP, H, and M antigens*

Virus strain	Viral antigens				Infectious virus titer	Hemadsorption
	Whole virus	NP	M	H		
Edmonston	4+[a]	4+	3+	3+	10^{6}[b]	+
LEC	4+	4+	3+	3+	$10^{7.5}$	+
Biken	4+	4+	1+	1+	$10^{3.5}$	+
IP-3	4+	4+	+/—	1+	10^{1}	+
DR	4+	4+	0	0	0	—

[a] Relative fluorescence intensity, see Materials and Methods
[b] $TCID_{50}$/0.1 ml

Production of infectious, cell-free virus by the variously infected cultures is shown in Table 1. The LEC strain was the most productive followed by the wild-type Edmonston strain. A considerable amount of cell-free virus was produced by the Biken strain. The IP-3 strain produced less virus than did the Biken strain, and the DR strain produced no detectable cell-free infectious viurs. Cultures infected with the Edmonston, LEC, Biken, and IP-3 strains of virus showed positive hemadsorption with monkey erythrocytes. In contrast, cells infected with the DR virus strain showed no adsorption of the erythrocytes.

Detection of Viral Antigens by Immune Fluorescence

Fluorescent antibody studies showed that the rabbit antisera were roughly equivalent to the mouse hybridoma products in their capacity to detect specific

antigens. Table 1 shows the results obtained using the rabbit antisera against separate structural components and a rabbit antiserum raised against purified whole virus. As expected, all five strains of virus produced large amounts of antigens detected by the antiserum against whole virus. Further, all strains produced high levels of viral NP antigen. The Biken virus strain clearly expressed the viral M antigen, whereas the IP-3 strain produced only trace amounts and the DR strain showed no evidence of M antigen expression. Both the Biken and IP-3 strains produced small amounts of the viral H antigen, but none was detected in cells infected with the DR strain. Both the viral M and H antigens were produced in large amounts by the Edmonston and LEC strains of virus.

Table 2. *Detection of measles virus antigens by immune fluorescence using hybridoma derived monoclonal antibodies*

Virus strain	Viral antigens				
	NP	P	H	F	M
Edmonston	4+	4+	3+	2+	2+
LEC	4+	4+	3+	1+	3+
Biken	4+	4+	2+	2+	1+
IP-3	4+	4+	3+	2+	0
DR	4+	4+	+/−	0	0

The mouse hybridoma antibodies detected various viral antigens as noted in Table 2. Viral NP and P antigens were brightly stained in cells infected with all five strains of virus. The H and F antigens were less bright, but clearly detected in the cultures infected with the Edmonston, LEC, Biken and IP-3 strains of virus. The staining of F antigen usually was much fainter than that of the H antigen. These antigens were undetectable or present in only trace amounts in cells infected with the DR strain of virus. Viral M antigen was clearly present in cells infected with the Edmonston, LEC, and Biken viruses, but was not detected in cells infected with either the IP-3 or DR strain of virus.

Distribution of Viral Antigens Within Infected Cells

The intracellular distribution of each antigen was similar in cells infected with all five strains of virus. Further, the antigen distributions were similar when either the monospecific rabbit antisera or the hybridoma antibodies were used. Viral NP and P antigens appeared as large cytoplasmic inclusions and as finely granular material, most commonly in a perinuclear location. They did not appear to be associated with cellular membranes. In agreement with earlier findings (15, 21), the NP antigen, but not the P antigen appeared in the cell nuclei as small punctate inclusions.

Viral H and F antigens had similar intracytoplasmic distributions with the antigens appearing as diffuse, finely granular material partly associated with the plasma membrane of the infected cell. Neither of these antigens was observed in the nuclei of infected cells. The viral M antigen was commonly expressed in infected cells as perinuclear inclusions or as diffuse granular material in the cytoplasm. Only a weak association with cellular membrane could be detected.

Discussion

While monoclonal antibodies are invaluable tools in the analysis of viral antigens, caution must be exercised in interpreting negative results of immunofluorescence, immunoprecipitation, and other methodologies employing these antibodies. Because of the site specificity of the monoclonal IgG molecules, minor variations in the structure of the viral polypeptides may lead to check the monoclonal antibodies no longer recognizing the antigen concerned. In this study monoclonal antibodies against two different antigenic determinants of each component (Sheshberadaran and Norrby, to be published) were used. The fact that comparable negative results were obtained with hyperimmune sera which contain antibodies to multiple epitopes on an antigenic molecule is strong evidence that the polypeptide is absent, altered, or diminished to sub-detectable levels.

The expression of measles virus antigens was variable in cells infected with Edmonston virus and four different strains of SSPE virus and the semi-quantitative variability was related to the titer of extracellular virus produced. In addition, characteristics of the cytopathic effects of the virus strains were, in part, related to the expression of the viral membrane glycoproteins. In contrast to Edmonston and LEC strains which produced high titers of extracellular virus and expressed all five viral antigens examined, the IP-3 and DR strains that produced little or no cell-free virus were deficient or lacking in the expression of one or more of the viral envelope antigens. The Biken strain which produced low, but significant, levels of cell-free virus expressed all five of the antigens, but the presence of H, F, and M antigen was notably decreased relative to the more productive strains (Tables 1 and 2).

Cells infected with all five strains of virus examined showed strong immunofluorescence for the NP and P antigens. A similar type of expression of these antigens has also been seen in cells infected with hamster brain adapted SSPE measles virus (HBS) and in HeLa cells chronically infected with Edmonston virus (15). The P viral antigen was localized within infected cells in the cytoplasmic inclusions in a pattern indistinguishable from the NP antigen, which suggests a close structural relationship between these antigens.

The expressions of the viral M, H and F antigens showed marked variability in cells infected with the various strains of virus and appeared to correlate with the production of extracellular virus and with the type of CPE induced in the infected cells. Cells infected with the LEC or Edmonston strains of virus showed bright M antigen immune fluorescence as intracytoplasmic inclusions and in some relation to the cell membranes. These virus strains also produced high levels of extracellular virus. Among the other virus strains, a correlation was seen between the level of M antigen expression and the production of the extracellular virus. The Biken strain which contained little M still produced significant amounts of virus while the IP-3 strain showed very little M and produced only trace amounts of virus.

The type of CPE induced by a virus strain appeared to correlate with the expressions of the viral F antigen. This protein is known to be the factor responsible for cell fusion (9). The Biken, IP-3, Edmonston, and LEC virus strains expressed F antigen and produced large, spreading syncytia in the infected cells.

In contrast, the DR virus infected cells expressed no detectable F antigen and developed small angular syncytia which rapidly degenerated and sloughed from the surface of the entire flask. Infectivity was, however, transferred to unfused cells at the periphery of the syncytia because new syncytia formed at the edge of the "hole" left by the sloughed syncytium.

The variability of expressions of the viral M antigen in cells infected with various strains of measles and SSPE virus is of interest since viral M protein is apparently poorly expressed in the brains of patients with SSPE (7, 8). With the four SSPE strains used in these studies, it is evident that M antigen expression can vary along a spectrum with the extremes being defined by high expression of the antigen in LEC virus and by no detectable expression in DR virus. This finding suggests that the antigen is subject to internal regulating mechanisms, the nature of which are unknown. In one study (18) it was proposed that the expression was controlled on the translation level rather than the transcription level. It is of interest that the expression of the viral M antigen can be regulated by host cell factors such as the intracellular levels of cAMP (14, 17). With such a potentially complex system of regulation for the viral M antigen as well as the H and F antigens which showed similar spectrums of expression, it must be noted that most virus strains, including the ones used in this study, available for study have received numerous passages in cell cultures. This fact makes it difficult to ascertain the role of such variability in human disease. For this reason the monoclonal antibodies are now being employed for identification of different measles virus antigens in the brains of patients with SSPE.

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References

1. Barbanti-Brodano, G., Oyanagi, S., Katz, M., Koprowski, H.: Presence of two different viral agents in brain cells of patients with subacute sclerosing panencephalitis. Proc. Soc. Exp. Biol. Med. **134**, 230—236 (1969).
2. Burnstein, F., Jacobsen, L. B., Zeman, W., Chen, T. T.: Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. Infect. Immun. **10**, 1378—1382 (1974).
3. Byington, D. P., Johnson, K. P.: Experimental subacute sclerosing panencephalitis in the hamster: Correlation of age with chronic inclusion-cell encephalitis. J. Infect. Dis. **126**, 18—26 (1972).
4. Carrigan, D. R., Johnson, K. P.: Experimental relapsing myelitis in hamsters associated with a variant of measles virus. In: Fields, B., Jaenisch, R., Fox, C. F. (eds.), Animal Virus Genetics. ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. XVIII, 759—768. New York: Academic Press 1980.
5. Carrigan, D. R., Johnson, K. P.: A mathematical model for the hemolysis of measles virus. J. Virol. Meth. **3**, 71—82 (1981).
6. Carrigan, D. R., McKendall, R. R., Johnson, K. P.: CNS disease following dissemination of SSPE measles virus from intraperitoneal inoculation of suckling hamsters. J. Med. Virol. **2**, 347—357 (1978).

7. HALL, W. W., CHOPPIN, P. W.: Evidence for lack of synthesis of the M polypeptide of measles virus in brain cells in subacute sclerosing panencephalitis. Virology **99**, 443—447 (1979).
8. HALL, W. W., CHOPPIN, P. W.: Measles virus proteins in the brain tissue of patients with subacute sclerosing panencephalitis: Absence of the M protein. N. Engl. J. Med. **304**, 1152—1155 (1981).
9. HARDWICK, J. M., BUSSELL, R. H.: Glycoproteins of measles virus under reducing and non-reducing conditions. J. Virol. **25**, 687—692 (1978).
10. JOHNSON, K. P., NORRBY, E.: Subacute sclerosing panencephalitis (SSPE) agent in hamsters. III. Induction of defective measles infection in hamster brain. Exp. Mol. Pathol. **21**, 166—178 (1974).
11. JOHNSON, K. P., NORRBY, E., SWOVELAND, P., CARRIGAN, D. R.: Experimental subacute sclerosing panencephalitis: Selective disappearance of measles virus matrix protein from the central nervous system. J. Infect. Dis. **144**, 161—169 (1981).
12. LIN, F. H., THORMAR, H.: Absence of M protein in a cell-associated subacute sclerosing panencephalitis virus. Nature **285**, 490—492 (1980).
13. MACHAMER, C. E., HAYES, E. C., ZWEERINK, H. J.: Cells infected with a cell-associated subacute sclerosing panencephalitis virus do not express M protein. Virology **108**, 515—520 (1981).
14. MILLER, C. A., CARRIGAN, D. R.: Reversible repression and activation of measles virus infection in neural cells. Proc. Nat. Acad. Sci. U.S.A. (in press, 1982).
15. NORRBY, E., CHEN, S., TOGASHI, T., SHESHBERADARAN, H., JOHNSON, K. P.: Five measles virus antigens demonstrated by use of mouse hybridoma antibodies in productively infected tissue culture cells. Arch. Virol. **71**, 1—11 (1982).
16. REED, L., MUENCH, M.: A simple method of estimating fifty percent endpoints. Amer. J. Hyg. **27**, 493—497 (1938).
17. ROBBINS, S. J., RAPP, F.: Inhibition of measles virus replication by cyclic AMP. Virology **106**, 317—326 (1980).
18. STEPHENSON, J. R., SIDDELL, S. G., TER MEULEN, V.: Persistent and lytic infections with SSPE virus: A comparison of the synthesis of virus-specific polypeptides. J. gen. Virol. **57**, 191—197.
19. THORMAR, H., JERVIN, G. A., KARL, S. C., BROWN, H. R.: Passage in ferrets of encephalitogenic cell-associated measles virus isolated from brain of a patient with subacute sclerosing panencephalitis. J. Infect. Dis. **127**, 678—685 (1973).
20. TOGASHI, T., ÖRVELL, C., VARTDAL, F., NORRBY, E.: Production of antibodies against measles virions by use of the mouse hybridoma technique. Arch. Virol. **67**, 149—157 (1981).
21. TYRRELL, J. L. D., RAFTER, J. D., ÖRVELL, C., NORRBY, E.: Isolation and characterization of the nucleocapsid and membrane proteins of measles virus. J. gen. Virol. **51**, 307—315 (1980).
22. UEDA, S., OKUNO, Y., HAMAMOTO, Y., OHYA, H.: Subacute sclerosing panencephalitis (SSPE): Isolation of a defective variant of measles virus from brain obtained at autopsy. Biken J. **18**, 113—122 (1975).

Authors' address: Dr. K. P. JOHNSON, Department of Neurology, University of Maryland Hospital, 22 S. Greene Street, Baltimore, MD 21201, U.S.A.

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