Infection of human oligodendroglioma cells by a recombinant measles virus expressing enhanced green fluorescent protein

Jonnie Plumb,1 W Paul Duprex,2 CH Stewart Cameron,3 Christiane Richter-Landsberg,4 Pierre Talbot,5 and Stephen McQuaid1

1Neuropathology Laboratory, Royal Group of Hospitals Trust, Belfast, Northern Ireland, United Kingdom; 2School of Biology and Biochemistry, The Queen’s University of Belfast, Belfast, Northern Ireland, United Kingdom; 3School of Medicine, The Queen’s University of Belfast, Belfast, Northern Ireland, United Kingdom; 4Department of Biology, University of Oldenberg, Oldenberg, Germany; and 5INRS-Institut Armand-Frappier, 531 Boulevard des Prairies, Laval, Quebec, Canada

One of the hallmarks of the human CNS disease subacute sclerosing panencephalitis (SSPE) is a high level of measles virus (MV) infection of oligodendrocytes. It is therefore surprising that there is only one previous report of MV infection of rat oligodendrocytes in culture and no reports of human oligodendrocyte infection in culture. In an attempt to develop a model system to study MV infection of oligodendrocytes, time-lapse confocal microscopy, immunocytochemistry, and electron microscopy (EM) were used to study infection of the human oligodendroglioma cell line, MO3.13. A rat oligodendrocyte cell line, OLN-93, was also studied as a control. MO3.13 cells were shown to be highly susceptible to MV infection and virus budding was observed from the surface of infected MO3.13 cells by EM. Analysis of the infection in real time and by immunocytochemistry revealed that virus spread occurred by cell-to-cell fusion and was also facilitated by virus transport in cell processes. MO3.13 cells were shown to express CD46, a MV receptor, but were negative for the recently discovered MV receptor, signaling leucocyte activation molecule (SLAM). Immunohistochemical studies on SSPE tissue sections demonstrated that CD46 was also expressed on populations of human oligodendrocytes. SLAM expression was not detected on oligodendrocytes. These studies, which are the first to show MV infection of human oligodendrocytes in culture, show that the cells are highly susceptible to MV infection and this model cell line has been used to further our understanding of MV spread in the CNS. Journal of NeuroVirology (2002) 8, 24–34.

Keywords: measles virus; oligodendrocytes; neuropathogenesis; subacute sclerosing panencephalitis

Introduction

Measles virus (MV) has been identified as the etiological agent of the human CNS infections subacute schizophrenic encephalitis [SSPE] (ter Meulen et al., 1983) and measles inclusion body encephalitis [MIBE] (Agamanolis et al., 1979). Both diseases are characterized by infection of neurons and glial cells after incubation periods ranging from months, MIBE, to years, SSPE, following the primary infection. As yet, the site of viral persistence within the body remains unknown (Rima et al., 1995). Restrictions of viral gene expression have been demonstrated in brain tissues from patients with both diseases affecting the genes encoding the matrix, fusion, and haemagglutinin proteins (Baczko et al., 1986; Cattaneo et al., 1988; Billeter and Cattaneo, 1991;
ter Meulen, 1997). Similar observations have been made in a rodent model of subacute measles encephalitis suggesting that transcriptional down-regulation of MV occurs in the early stages of infection (Schneider-Schaulies et al, 1989).

Neuropathological and immunopathological studies on autopsy tissue isolated from individuals with SSPE have established that gray matter neurons and their processes are infected at high levels and that many oligodendrocytes are infected in the white matter (Budka et al, 1982; Allen et al, 1996). Viral antigen is detected within both the nuclei and cytoplasm of oligodendrocytes in SSPE brain tissue (Allen et al, 1996). Although the infection is widespread in the hemispheric white matter, relatively low numbers of infected oligodendrocytes are observed in the brain stem and spinal cord. Astrocytes are also infected throughout the CNS but to a much lesser degree. The virus is distributed throughout the CNS, from the temporal and frontal cortices, to the medulla, pons, and cervical spinal cord regions. In some cases low numbers of antigen positive neurons have been observed in the cerebellum (Allen et al, 1996). Such studies have suggested transynaptic spread of the virus in a cephalo-caudal direction. Observations using the differentiated human neuron cell line, NT2, have given support to the hypothesis of transsynaptic spread (Lawrence et al, 2000) but as yet the mechanism has not been elucidated. It is also unclear how the neurons or oligodendrocytes initially become infected or how the infection is propagated through the white matter.

Some recent studies on cultured astrocytes and neurons have shown the importance of extended cell processes in cell-to-cell spread of virus. Cultured human astrocytoma cells have intimately associated extended cell processes that are utilized by MV in the infection of surrounding cells (Duprex et al, 1999). Studies have also shown that cultured human neurons can become infected via the extended processes that are in contact with more readily infected neuroepithelial cells in mixed cell populations (McQuaid et al, 1998). Such a mechanism may be due to the presence of virus proteins in the neuroepithelial cell plasma membrane where it contacts the neurons. Furthermore, it has also been shown that viral spread in cultured neurons occurs in the absence of syncytium formation and with minimal extracellular virus production (Lawrence et al, 2000).

Nearly all of the published work on the susceptibility of CNS cells to MV infection have utilized astrocytic and, to a lesser extent, neuronal cell lines (Miller and Carrigan, 1982; Schneider-Schaulies et al, 1990, 1993; McQuaid et al, 1998; Duprex et al, 1999, 2000; Lawrence et al, 2000). Studies on glial cell lines have indicated that, in contrast to nonneural cells, MV transcription can be down-regulated by intrinsic host cell factors, whereas the differentiation state of the cells influences the translation of virus proteins (Schneider-Schaulies et al, 1993). Despite these investigations there are very few studies that have utilized either primary cells or oligodendrocyte cell lines to study MV infection in this important cell type. In one previous report, Atkins et al (1991) reported that a nonrodent-adapted strain of Edmonston strain of MV multiplied and produced a cytopathic effect in primary cultures of rat oligodendrocytes (Atkins et al, 1991). Viral infection in that study was only monitored by cytopathic effects and no viral immunocytochemistry or ultrastructural investigations were undertaken.

In this study, we aimed to establish a model of MV infection of oligodendrocytes and to utilize this model to determine if the virus infection was propagated via cell processes. MO3.13 is an immortal human-human hybrid derived by lectin-enhanced, polyethylene glycol-mediated somatic cell fusion between the thioguanine-resistant rhabdomyosarcoma mutant RD-TG.6 and primary human oligodendrocytes obtained from cultures of adult temporal lobectomies (Talbot et al, 1993; Ursell et al, 1995). MO3.13 cells are characterized as being positive by immunocytochemistry and Western blotting for the oligodendrocyte specific markers myelin basic protein (MBP) and proteolipid protein (PLP) (McLaurin et al, 1995). The OLN-93 cell line was established from primary cultures of glial cells prepared from the brains of 1-day-old Wistar rats (Richter-Landsberg and Heinrich, 1996). In some recently published studies (Duprex et al, 1999, 2000), we have come to appreciate the usefulness of a recombinant MV, which expresses enhanced green fluorescence protein (EGFP). This virus was therefore chosen to examine oligodendrocyte cell infection and virus spread from cell-to-cell. The MO3.13 cell line was also analyzed for expression of a receptor used by vaccine strains of MV, CD46 (Naniche et al, 1993) and for another recently described MV receptor SLAM (Tatsuo et al, 2000). In parallel investigations snap-frozen blocks of white matter from an autopsy case of SSPE were examined for the expression of CD46 and SLAM by immunocytochemistry.

Results

Characterization and expression of MV receptors on MO3.13 cells
At the split ratios used, MO3.13 and OLN-93 cells were highly proliferative and attained confluence within 3 days. Both cell lines expressed markers indicative of oligodendrocyte lineage (Figure 1A, B). Unfixed MO3.13 cells were examined for the presence of CD46 by indirect immunofluorescence. Expression of CD46 was present in localized patches on the cell surface of all cells (Figure 1C). MO3.13 cells were negative for SLAM expression (Figure 1D). As expected, the B-cell line B95a showed SLAM expression on all cells (Figure 1D1).
Figure 1 Immunoreactivity in MO3.13 cells, OLN-93 cells, and oligodendrocytes in SSPE. The fluorescent images are all composite confocal sections through 8–10 μm and cell nuclei are counterstained with propidium iodide. (A) Expression of oligodendrocyte-specific protein galactocerebroside on the surface of uninfected MO3.13 cells. (B) Oligodendrocyte-specific proteolipid protein expression on the surface of uninfected OLN-93 cells. (C) CD46 expression at the cell surface of MO3.13 cells. (D) Lack of expression of the MV receptor SLAM on MO3.13 cells. The insert shows typical SLAM expression on the surface of B95 cells. (E) CD46 is present on cerebral endothelium (arrow) and cells with the morphological characteristics of oligodendrocytes in the white matter in SSPE tissue sections. (F) SLAM expression on cells within the perivascular cuff in SSPE. Note the absence of expression in the surrounding parenchyma. (G) Detection of MV antigen in a chain of interfascicular oligodendrocytes in the white matter in SSPE. The photomicrograph was taken with differential interference contrast microscopy on a Leica Aristoplan microscope. (Magnification: A–D, X400; E–G, X250.)
Immunohistochemical staining of MV receptors in SSPE tissue
To extend these in vitro observations SSPE tissue sections were immunocytochemically stained for CD46 and SLAM. CD46 was expressed on all cerebral endothelium and on subpopulations of cells in the white matter with the morphological characteristics of oligodendrocytes (Figure 1E). Small numbers of neurons in the gray matter were also observed to express CD46. SLAM was absent from cells in the parenchyma of the brain but was expressed on cells within the perivascular infiltrates surrounding blood vessels (Figure 1F, arrow). SSPE tissue sections were immunohistochemically stained with MAb to measles virus nucleocapsid (N). Based on morphological criteria neurons in the gray matter and oligodendrocytes in the white matter were the cells types predominantly infected with MV from the nine cases of SSPE studied (Allen et al., 1996). In very rare instances chains of MV-infected interfascicular oligodendrocytes were observed in the white matter (Figure 1G). No viral antigen was detectable in processes between such groups of cells. The areas surrounding such chains of cells also contained scattered MV-positive oligodendrocytes (data not shown).

Infection of oligodendrocytes with MVeGFP
To establish an in vivo model of MV infection of oligodendrocytes, MO3.13 cells were infected with MVeGFP. The cells were readily infectable and up to 90% of cells became infected 48 h postinfection (h.p.i.). EGFP autofluorescence was observed in the cell bodies and fine processes of the infected cells. Infectious virus was recovered from both the supernatant and cell-associated components. MVeGFP was cultured by five passages on MO3.13 cells at an MOI of 0.01. Throughout this process, infected cells were evident by GFP fluorescence 24 h.p.i. and more than 90% of the cells were infected by 72 to 96 h.p.i. Titres of MVeGFP(MO3.13p5) were obtained in triplicate by TCID50 assay on Vero and MO3.13 cells. During this time, viral titres did not change (Table 1), indicating that the virus did not significantly adapt to MO3.13 cells. By comparison OLN-93 cells growing on 25-cm² flasks or glass coverslips could only be infected with MVeGFP at a very low level. EGFP autofluorescence was observed in small clusters of infected OLN-93 cells (approxi-

Table 1 Titres of MVeGFP (verop1) and MVeGFP (MO3.13p5) viruses obtained following their growth on MO3.13 and Vero cells

| Cell line | MVeGFP(verop1) titre (50% TCID50/ml) | MVeGFP(MO3.13p5) titre (50% TCID50/ml) |
|-----------|--------------------------------------|---------------------------------------|
| MO3.13    | 2.45 x 10⁴                           | 1.2 x 10⁴                             |
| Vero      | 3.16 x 10⁴                           | 2.14 x 10⁴                             |

Titres were measured as 50% tissue culture infectious doses/ml and are the averages obtained from a triplicate of each experiment.

Localization of viral antigen within infected MO3.13 cells
All experiments were carried out using MO3.13 cells infected with MVeGFP (MO3.13p5) virus at an MOI of 0.01. Immunocytochemistry with an anti-measles virus N MAb on cells at 48 h.p.i. showed the presence of cytoplasmic antigen in the presence of MVeGFP autofluorescence (Figure 2A). Infected cells expressed EGFP in the presence of MV nucleocapsid, detected with a MAb and visualized with Alexa 568 goat anti-mouse. A clear localization of EGFP to the nucleus of infected cells was observed as previously noted in other cell types. Such accumulation of EGFP in the nucleus only takes place in infected cells and at present is assumed to occur by a nonspecific mechanism (Duprex et al., 1999). The punctate nature of the nucleocapsid staining is typical of MV-induced intracytoplasmic inclusion bodies. It is also noteworthy that EGFP autofluorescence can be detected in cells that are negative for viral antigen (Figure 2A, arrow b) confirming previous observations and indicating that utilization of the recombinant virus is a very sensitive means to detect virus-infected cells.

On acetone-fixed cells at 48 h.p.i. MV antigens were detected using an SSPE serum, which predominantly detects N and phosphoprotein proteins, visualized by a FITC-conjugated rabbit anti-human secondary antibody. The intermediate filament, vimentin, was used as a counterstain to show both uninfected and infected cells. Vimentin was detected using a MAb visualized by an Alexa 568-conjugated goat anti-mouse secondary antibody. Once again large accumulations of nucleocapsid were seen in the cytoplasm of infected cells (Figure 2B inset). Antigen was also detected in the connecting processes between cells (Figure 2B, arrows) and in the fine, branching processes of singly infected cells (Figure 2B, asterisk).

Ultrastructural analysis of MV-infected MO3.13 cells
Monolayers of MO3.13 cells growing on 25-cm³ flasks, infected at an MOI of 0.01, were fixed and processed for ultrastructural analysis. A high percentage of the cells viewed by EM were infected as evidenced by an abundance of spherical nucleocapsid inclusion bodies dispersed or aggregated throughout the cytoplasm (Figure 2C, dashed circle). This is consistent with the immunocytochemical data (Figure 2B, inset). Figure 2D is representative of nucleocapsids in cross-section observed to underlie...
Figure 2  Immunocytochemical and ultrastructural analysis of MVeGFP-infected MO3.13 cells. (A) MV nucleocapsid (red) and GFP (green) expression in MO3.13 cells. Arrow a indicates expression of GFP- and MV-antigen in the same cell. Arrow b indicates a MVeGFP-infected cell that is negative for nucleocapsid. (B) Nucleocapsid and phosphoprotein antigens, detected with SSPE antisera (green) and the intermediate filament vimentin (orange) in MO3.13 cells. Large accumulations of nucleocapsids are present in the cytoplasm of infected cells (insert B1) and in the connecting processes between cells (arrows). (C) Spherical mass of viral inclusions in the cytoplasm (magnification × 20250, encircled). (D–F) Various stages of the budding of MV virions from the surface of MO3.13 cells. (D) Protein thickening indicative of the budding process (magnification × 48500, arrows). (E) MV budding (arrows) at the cell surface (magnification × 24000). (F) Mature MV virion displaying nucleocapsid (arrows) dispersed within a thickened envelope membrane (magnification × 71000). The EM images were produced from EM negatives scanned into ADOBE Photoshop.
cell membranes displaying modification or thickening (arrows) that are typical of the MV budding structures that form and release mature MV virions. The membranes of MO3.13 cells were examined at greater magnifications to identify areas of membrane thickening or modification and evidence of MV budding. Different stages of virus budding were observed along the membranes. In Figure 2E, the photomicrograph demonstrates a typical infected MO3.13 cell with five mature MV virions (arrows) at the cell surface. The formation of a virus bud begins with thickening of cell membranes, which project outwards from the cell until it pinches off to form a mature MV virion with characteristic surface projections (Figure 2F, arrows).

Cell-to-cell spread of MV in oligodendrocytes

Having established the MO3.13 cell line as a suitable model, we wished to examine the spread of MV in oligodendrocytes. MO3.13 cells were infected at a MOI of 0.01 with MVeGFP and infected areas observed regularly by UV microscopy from 24 h.p.i. Two representative time courses of MVeGFP-infected cells that illustrate both the spread of virus along cell processes and the fusion of virus-infected cell bodies are shown in Figures 3A and B. In Figure 3A, virus spread is mediated via interconnecting cell processes. At 24.75 h.p.i., EGFP is present in a cell process (arrow) of an infected cell. Within 15 min, GFP is present in the cell body of a neighbouring cell. By 26.5 h.p.i., the infection has progressed to adjacent cells. Figure 3B illustrates an example of fusion-mediated spread of MVeGFP. Three neighbouring small syncytia (27 h.p.i., arrows) fuse together over a 3.5-h time period, forming a larger syncytium. By 96 h.p.i., processes and fusion-mediated spread of virus led to infection of the complete cell monolayer (Figure 3A, insert). Cytopathic effect due to syncytia formation was observed throughout the monolayer.

Discussion

In the present study, we have demonstrated that MV is capable of infecting the human oligodendroglial cell line MO3.13. This is the first report of MV infection of a human oligodendrocyte cell line, an important cell type known to be infected in SSPE. The cells are readily infectible by a recombinant MV and infectious virus was produced. By comparison, the rodent oligodendrocyte cell line, OLN-93 displayed a low susceptibility to MVeGFP infection and infectious virus was not released. MO3.13 cells expressed high levels of cell surface CD46 but were negative for the recently described MV receptor SLAM. This is consistent with the observation in SSPE tissue sections where CD46 but not SLAM expression was detected on oligodendrocytes in the white matter. However, the numbers of CD46-positive oligodendrocytes detected by immunohistochemistry was only a small percentage of the total number of oligodendrocytes in the white matter of the brain areas examined. A more detailed description of CD46 and SLAM expression in the normal and MV-infected CNS and peripheral tissues is currently in preparation (McQuaid et al, unpublished results). It has been shown that not all cells that are susceptible to MV infection express detectable levels of CD46 (Yanagi et al, 1994; Dunster et al, 1995; Horvat et al, 1996), and SLAM is only constitutively expressed on immature thymocytes, CD45RO⁺ memory T cells, and a proportion of B cells (Sidorenko and Clarke, 1993; Cocks et al, 1995). The mechanism of MV entry into these cells remains unclear and would seem to indicate that other MV receptors may exist on mammalian cells.

In a previous study, we made use of a human astrocytoma cell line to observe virus spread using a recombinant EGFP-expressing MV virus (Duprex et al, 1999). Individual infected cells, identified by EGFP autofluorescence, were monitored by CSLM and the virus spread was shown to be cell process-mediated with a rapid progression of GFP from cell-to-cell. Utilizing this technique, MO3.13-propagated MVeGFP virus was observed to spread in MO3.13 cell monolayers. Virus propagation through the monolayer was observed to be predominantly a cell process-mediated event. The actual mechanism of virus spread from an infected cell process to an adjacent uninfected cell process has not been established in these studies. It is possible that microfusion of cell processes occurs with the accompanying passage of viral ribonucleoprotein into an uninfected cell process. However, infected cells were also observed to spread infection by cell body-to-cell body fusion. Adjacent cells become infected and then fuse to form syncytia. These observations have previously been made in MVeGFP-infected murine neuroblastoma cells where virus spreads from cell-to-cell both by fusion and via cell processes (Duprex et al, 2000).

MV infection of alpha/beta interferon receptor-defective mice expressing human CD46 has been used to suggest that replication is much more efficient in the rodent CNS than the peripheral nervous system, with the virus propagating mostly in the easily accessible ependymal cells (Mrkic et al, 1998). Viral RNA or antigen was often detected in contiguous cells, suggesting that in the brain of transgenic mice MV propagation may be based largely on lateral cell-to-cell contacts. This demonstrated both in vivo and in vitro, MV spread in the CNS most likely involves localized fusion events at cell-to-cell contact points without the requirement for specific viral receptor(s) (McQuaid et al, 1998; Duprex et al, 1999; Lawrence et al, 2000). Similarly, in the present study we have shown that human oligodendroglioma cells in culture spread MV infection both by fusion and along interconnecting processes.

Previous ultrastructural investigations of MV infected astrocyte and neuronal cell lines have demonstrated viral nucleocapsids dispersed or in small
Figure 3  Cell-to-cell spread of MVeGFP in MO3.13 cells (see text for details). MO3.13 cells were infected with MVeGFP at an MOI of 0.01. Single or small numbers of infected cells were identified in the monolayer by UV microscopy and the time-lapse facility of the confocal system was used to acquire Z-series images every 20 min over a 48-h time period. Two representative time-course experiments are shown (A and B). Series A demonstrates process-mediated spread of virus from cell-to-cell. Series B demonstrates the fusion of three infected syncytia (arrows) over a 3.5-h time period. The insert at the bottom of series A demonstrates the extent of infection by 96 h postinfection. The images were collected in multiple optical sections through the entire thickness of the cells in single-excitation mode. The number of hours postinfection at which each autofluorescent image set was collected is indicated. EGFP autofluorescence is shown in false-white color. (Magnification × 100.)
clumps throughout the cytoplasm (Macintyre and Armstrong 1976; McQuaid et al., 1998; Lawrence et al., 2000). EM observations of astroglial cultures displayed MV in various stages of assembly and extracellular virions were routinely observed. These mature virions have surface projections, thickened envelope membranes, and the nucleocapsid tubules are observed to be dispersed peripherally in a spiral form (Macintyre and Armstrong, 1976). Electron microscopy of undifferentiated and differentiated NT2 cells showed that viral budding occurred very rarely in undifferentiated cells and was not observed on the surface of differentiated NT2 cells (McQuaid et al., 1998; Lawrence et al., 2000). However, nucleocapsids were aligned at the cell membrane of differentiated NT2 cells, in neuronal processes and at presynaptic neuronal membranes. Ultrastructural analysis of MV-infected MO3.13 cells revealed an abundance of MV nucleocapsid within the cytoplasm, both in isolation and as aggregates. Typical plasma membrane modifications and various stages of budding (Fleury et al., 1980) were also observed consistent with the productive nature of MV infection from MO3.13 cells.

This pattern of MV nucleocapsid localization mirrored observations made with single- and dual-labeled immunofluorescence of MVeGFPp5-infected MO3.13 cells. MV nucleocapsid was observed throughout the cell cytoplasm with dense clumps of virus evident in the perinuclear regions. When dual-labeled for MV antigens and EGFP, it was evident that infected MO3.13 cells expressed GFP autofluorescence in the absence of detectable MV antigen. Such observations have led to the conclusion that EGFP expression provides an early indicator of MV infection in vitro (Duprex et al., 1999).

In the adult CNS, fully differentiated interfascicular oligodendrocytes occur in the white matter and are characterized by their many connections to segments (internodes) of myelin sheaths wrapped around axons. The processes, which link the oligodendrocyte cell body to the sheath, are narrow and tortuous (Knobler et al., 1974). The paranod al regions of oligodendrocyte can also contact other glial cells of the CNS such as astrocyte by gap junctions, indicating functional coupling (Berry et al., 1995). Gap junctions establish intracellular channels of communication through which ions and small solutes (<1300 D) can pass (Mugnaini, 1982). However, it is unlikely that viral nucleocapsid could pass from cell-to-cell via intact gap junctions. Occasionally interfascicular oligodendrocytes may be seen aligned in rows. Where this occurs, the cell membranes of adjacent cells are in intimate contact but lack specific junctional contacts. In very rare examples, in the white matter from cases of SSPE, chains of interfascicular oligodendrocytes were demonstrated to have detectable levels of MV. However, in such autopsy tissue, fixed for light microscopy, it is impossible to determine if virus is present in the very fine oligodendrocyte processes in the white matter. Oligodendrocytes also occur in the gray matter as perineuronal satellite cells. Individual satellite cells are thought to be in close contact with single neurons. In some pathological situations, such cells have been described to have processes extending to myelin sheaths (Ludwin, 1979). The extent of oligodendrocyte processes in the CNS and the findings, in the present study, of extensive infection of oligodendrocyte processes in vitro raises the possibility of oligodendrocyte-to-neuron infection in the CNS as a means of initiating spread of virus throughout the CNS. It is postulated that, once present in neurons, MV can spread transneuronally throughout the CNS (Allen et al., 1996; Lawrence et al., 2000) possibly by fast axonal transport (Oldstone et al., 1999).

Like a small number of other viruses, MV has been identified as potentially persisting in the CNS (Liebert, 1997). For example, Borna virus causes CNS disease in several species, and recent studies have suggested a potential role for these viruses in human mental health (Gonzalez-Dunia et al., 1997). The polyomavirus JC, which infects oligodendrocytes in vivo, is associated with most cases of progressive multifocal leukoencephalopathy, a demyelinating disease of the CNS leading to death within months of first presentation (Askamit, 1995; Eggers et al., 1999). However, the specific cell type or neuroanatomical location(s) of CNS viral persistence remains unknown. The finding that MO3.13 cells can sustain persistent coronavirus infections (Arbour et al., 1999a, 1999b) raises the possibility that other viruses, such as measles, may persist in oligodendrocytes in vivo.

A logical progression of these experiments will be to use primary oligodendrocyte cultures (McCarthy and De Vellis, 1980; Gates et al, 1985) to analyze virus spread. It would also be important to analyze CD46/SLAM expression on mature primary oligodendrocytes.

Materials and methods

Cell lines

Two cells lines of oligodendrocyte lineage, human MO3.13 (Talbot et al, 1993) and rat OLN-93 (Richter-Landsberg and Heinrich, 1996), were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS), 0.1% penicillin and streptomycin, and 4 mM glutamine in 25-cm³ flasks at 37°C with 5% (MO3.13) or 10% (OLN-93) CO₂ added. OLN-93 cells have been shown to express the oligodendrocyte-specific markers, galactocerebroside, MBP, PLP, and myelin-associated glycoprotein (MAG) (Richter-Landsberg and Heinrich, 1996; Strelau and Unsicker, 1999). When confluent, cells were passaged at a 10:1 (MO3.13) or 20:1 (OLN-93) split ratio into 25-cm³ flasks.
Virus infection of cells
MO3.13 and OLN-93 monolayers grown in 25-cm² flasks were infected with adapted MVeGFP virus that had previously been grown on Vero cells (Duprex et al., 1999). Cell monolayers (60% confluency) were rinsed with maintenance medium (D MEM supplemented with 2% FCS) and cells were infected with virus at a multiplicity of infection (MOI) of 0.01 and incubated for 1 h at 37°C. After this time unadsorbed virus was removed, maintenance medium was added, and cells were incubated at 37°C for varying time periods. Cell sheets were monitored by UV microscopy (Leica) daily for the appearance of M VeGFP positive cells. For adaptation of M VeGFP to oligodendrocyte cell lines, heavily infected monolayers (>90%) were freeze-thawed three times. Virus stocks were stored at −70°C. Virus titres were determined by TC ID ₅₀ (Reed and Muench, 1938), and the stocks were diluted in PBS and added to the coverslips and incubated for 1 h at 37°C. Coverslips dual-labeled with SSPE serum and vimentin were subsequently incubated in rabbit anti-human FITC and Alexa 568 goat anti-mouse. Coverslips were washed twice in PBS and mounted using Citiflour.

A Leica TCS/NT confocal scanning laser microscope (C SL M) equipped with a krypton/argon laser was used to examine the samples for fluorescence. Alexa 488-labelled samples or M VeGFP autofluorescence was visualized by excitation at 488 nm with a 506–538 band-pass emission filter. Alexa 568-labelled samples were imaged by excitation at 568 nm with a 564–596 band-pass emission filter.

Vital confocal fluorescence microscopy
Cells were grown to 60% confluence in 25-cm² tissue culture flasks. Cells were infected at an MOI of 0.01 with M VeGFP. As previously described, an inverted UV microscope was used to monitor the monolayers for the appearance of infected cells (Duprex et al., 1999). In initial experiments, flasks were oriented on the microscope stage and marked to permit the repeated observation of chosen groups of infected cells in the monolayers. Observations were made over a period of 24 h at hourly intervals. In additional experiments singly infected or infected groups of cells were selected and the time-lapse ability of the confocal system was used to acquire Z-series images every 20 min over 48 h (Duprex and Rima, 2001).

Ultrastructural analysis of cells
Infected MO3.13 cells were fixed and embedded for ultrastructural analysis. Monolayers were fixed in 2.5% glutaraldehyde for 90 min at 4°C, then rinsed in 0.2 M cacodylate buffer for 30 min at 4°C. Cells were then postfixed for 1 h at room temperature in 2% osmium tetroxide and rinsed in distilled water. Monolayers were dehydrated in graded alcohols and propylene oxide was used to detach the cells from the flask. Cells were subsequently embedded in Agar 100 embedding resin as previously described (McCormack et al., 1983). Semithin sections were stained in toluidine blue to identify areas with high numbers of cells and ultrathin sections (90 nm) were cut from these regions. Sections were lifted onto copper EM grids and stained with uranyl acetate and lead citrate. Sections were
examined on a Hitachi H-600 transmission electron microscope.

**Immunohistochemical staining of SSPE tissue**

Cryostat sections (12 μm) were cut from snap-frozen tissues from a SSPE case and fixed in 10% formalin. After blocking endogenous peroxidase in 0.5% H2O2 in methanol for 10 min, sections were incubated in polyclonal anti-CD46 or MAb to SLAM overnight at 4°C. Furthermore, selected blocks of predominantly white matter tissue from nine autopsy cases of SSPE were immunohistochemically stained for MV antigens as described previously (Allen et al. 1996). Bound receptor or viral antibodies were detected using diaminobenzidine or aminoethylcarbazole, as peroxidase substrate. Sections were counterstained with haematoxylin. Photomicroscopy was carried out on a Leitz Aristoplan fitted with differential interference contrast.

**References**

Agamanolis DP, Tan JS, Parker DL (1979). Immunosuppressive measles encephalitis in a patient with a renal transplant. *Arch Neurol* 36: 686–690.

Allen IV, McQuaid S, McMahon J, Kirk J, McConnell R (1996). The significance of measles virus antigen and genome distribution in the CNS in SSPE for mechanisms of viral spread and demyelination. *J Neuropathol Exp Neurol* 55: 471–480.

Arbour N, Cote G, Lachance C, Chagnon F, Tardieu M, Cashman NR, Talbot PJ (1999). Acute and persistent infection of human neural cell lines by human coronavirus OC43. *J Virol* 73: 3350–3358.

Arbour N, Ekande S, Cote G, Lachance C, Chagnon F, Tardieu M, Cashman NR, Talbot PJ (1999). Persistent infection of human oligodendrocytic and neuroglial cell lines by human coronavirus 229E. *J Virol* 73: 3326–3337.

Askamit AR Jr (1995). Progressive multifocal leucoencephalopathy: a review of the pathology and pathogenesis. *Microsc Res Tech* 32: 302–311.

Atkins GJ, Mooney DA, Fahy DA, Ng SH, Sheahan BJ (1991). Multiplication of rubella and measles viruses in primary rat neural cell cultures: relevance to a postulated triggering mechanism for multiple sclerosis. *Neuropathol Appl Neurobiol* 17: 299–308.

Baczko K, Liebert UG, Billette MA, Cattaneo R, Budka H, ter Meulen V (1986). Expression of defective measles virus genes in brain tissues of patients with subacute sclerosing panencephalitis. *J Virol* 59: 472–478.

Berry M, Bannister LH, Standring SM (1995). Nervous system. In: Grays Anatomy: The Anatomical Basis of Medicine and Surgery, 38 ed. Berry MM, Standring SM, Bannister LH (eds). Churchill Livingstone: London, pp 901–1397.

Billette MA, Cattaneo R (1991). Molecular biology of defective measles viruses persisting in the human central nervous system. In: *The Paramyxoviruses*, Kingsbury D (ed). Plenum Press: New York, pp 323–345.

Budka H, Lassmann H, Popow-Kraupp T (1982). Measles virus antigen in panencephalitis. An immunmorphological study stressing dendritic involvement in SSPE. *Acta Neuropathol (Berl)* 56: 52–62.

Cattaneo R, Schmid A, Eschle D, Baczko K, ter Meulen V, Billette MA (1988). Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. *Cell* 55: 253–265.

Cocks BG, Chang CC, Carballido JM, Yssel H, de Vries JE, Aversa G (1995). A novel receptor involved in T-cell activation. *Nature* 376: 260–263.

Dunster LM, Schneider-Schaulies J, Dehoff MH, Holers VM, Schwartz-Albiez R, ter Meulen V (1995). Moesin, and not the murine functional homologue (Crry/p65) of human membrane cofactor protein (CD46), is involved in the entry of measles virus (strain Edmonston) into susceptible murine cell lines. *J Gen Virol* 76: 2085–2089.

Duprex WP, McQuaid S, Hangartner L, Billette MA, Rima BK (1999). Observation of measles virus cell-to-cell spread in astrocytoma cells by using a green fluorescent-protein expressing recombinant virus. *J Virol* 73: 9568–9575.

Duprex WP, McQuaid S, Roscic-Mrkic M, Cattaneo R, McCallister C, Rima BK (2000). *In vitro* and *in vivo* infection of neural cells by a recombinant measles virus expressing enhanced green fluorescent protein. *J Virol* 74: 7972–7979.

Duprex WP, Rima BK (2001). Green-fluorescent protein. Measles virus cell to cell spread; real-time visualisations. In: *Methods in Molecular Biology*. Hicks BW (ed). Humana Press: New York, in press.

Eggers C, Stellbrink H, Buik T, Dorries K (1999). Quantification of JC virus DNA in CSF of patients with HIV associated PML: a longitudinal study. *J Infect Dis* 180: 1590–1604.

Fleury H, Bonnez W, Pometan JP, du Pasquier P (1980). Differences in early ultrastructural aspects of the replication of measles and subacute sclerosing panencephalitis viruses in a cell culture from a human astrocytoma. *J Neuropathol Exp Neurol* 39: 131–137.

Gates M, Sheahan BJ, O’Sullivan M, Atkins G (1985). The pathogenicity of the A7, M9 and L10 strains of Semliki Forest virus for weanling mice and primary mouse brain cell cultures. *J Gen Virol* 66: 2365–2373.

Gonzalez-Dunia D, Sauder C, de la Torre JC (1997). Borna disease virus for weaning mice and primary mouse brain cell cultures. *J Gen Virol* 78: 686–690.

Horvat B, Rivailier F, Varion-Krishnan G, Cardoso A, Gerlier D, Baroudin-Combe C (1996). Transgenic mice expressing human measles virus (MV) receptor CD46 provide cells exhibiting different permissivities to MV infections. *J Virol* 70: 6673–6681.

Knochler RL, Stempak JG, Laurencin M (1974). Oligodendroglial ensheathment of axons during myelination in the developing rat central nervous system. A serial section electron microscopical study. *Ultrastructural Res* 49: 34–49.

Lawrence DM, Patterson CE, Gales TL, D’Orazio JL, Vaughn MM, Rall GF (2000). Measles virus spread between neurons requires cell contact but not CD46 expression, syncytium formation, or extracellular virus production. *J Virol* 74: 1908–1918.
Measles virus infection of oligodendroglial cell lines

Rima BK, Earle JA, Yeo RP, Herlihy L, Baczko K, ter Meulen V, Carabana J, Caballero M, Celma ML, Fernandez-Munoz R (1995). Temporal and geographical distribution of measles virus genotypes. J Gen Virol 76: 1173–1180.

Schneider-Schaulies S, Liebert UG, Baczko K, Cattaneo R, Billeter MA, ter Meulen V (1989). Restriction of measles virus gene expression in acute and subacute encephalitis of Lewis rats. Virology 171: 525–534.

Schneider-Schaulies S, Liebert UG, Baczko K, ter Meulen V (1990). Restricted expression of measles virus in primary rat astroglial cells. Virology 177: 802–806.

Schneider-Schaulies S, Schneider-Schaulies J, Bayer M, Loffler S, ter Meulen V (1993). Spontaneous and differentiation-dependent regulation of measles virus gene expression in human glial cells. J Virol 67: 3375–3383.

Sidorenko SP, Clark EA (1993). Characterization of a cell surface glycoprotein IPO-3, expressed on activated human B and T lymphocytes. J Immunol 151: 4614–4624.

Strelau J, Unsicker K (1999). Expression and function in two oligodendroglial cell lines representing distinct stages of oligodendroglial development. Glia 26: 291–301.

Talbot PJ, Ekande S, Cashman NR, Mounir S, Stewart JN (1993). Neurotropism of human coronavirus 229E. Adv Exp Med Biol 342: 339–346.

Tatsuo H, Ono N, Tanaka K, Yanagi Y (2000). SLAM (CDw150) is a cellular receptor for measles virus. J Virol 74: 7420–7427.

Mugnaini E (1982). Membrane specializations in neuroglial cells and at neuron-glia contacts. In: Neuronal-glial cell Interrelationships. Sears TA (ed). Springer-Verlag: Berlin, pp 39–56.

Naniche D, Varior-Krishnan G, Cervoni F, Wild TF, Rossi B, Rabourdin-Combe C, Gerlier D (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol 67: 6025–6032.

Rima BK, Earle JA, Yeo RP, Herlihy L, Baczko K, ter Meulen V, Carabana J, Caballero M, Celma ML, Fernandez-Munoz R (1995). Temporal and geographical distribution of measles virus genotypes. J Gen Virol 76: 1173–1180.

McQuaid S, Campbell S, Wallace IJ, Kirk J, Cosby SL (1998). McLaughlin J, Trudel GC, Shaw IT, Antel JP, Cashman NR (1995). A human glial hybrid cell line differentially expressing genes subserving oligodendrocyte and astrocyte phenotype. J Neurobiol 26: 283–293.

Mugnaini E (1982). Membrane specializations in neuroglial cells and at neuron-glia contacts. In: Neuronal-glial cell Interrelationships. Sears TA (ed). Springer-Verlag: Berlin, pp 39–56.

Naniche D, Varior-Krishnan G, Cervoni F, Wild TF, Rossi B, Rabourdin-Combe C, Gerlier D (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol 67: 6025–6032.

Oldstone MB, Lewicki H, Thomas D, Tishon A, Dales S, Patterson J, Manchester M, Homann D, Naniche D, Holz A (1999). Measles virus infection in a transgenic model: virus-induced immunosuppression and central nervous system disease. Cell 98: 629–640.

Richter-Landsberg C, Heinrich MJ (1996). OLN-93: a new permanent oligodendroglia cell line derived from primary rat brain glial cultures. Neurosci Res 45: 161–173.

Naniche D, Varior-Krishnan G, Cervoni F, Wild TF, Rossi B, Rabourdin-Combe C, Gerlier D (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol 67: 6025–6032.

Oldstone MB, Lewicki H, Thomas D, Tishon A, Dales S, Patterson J, Manchester M, Homann D, Naniche D, Holz A (1999). Measles virus infection in a transgenic model: virus-induced immunosuppression and central nervous system disease. Cell 98: 629–640.

Richter-Landsberg C, Heinrich MJ (1996). OLN-93: a new permanent oligodendroglia cell line derived from primary rat brain glial cultures. Neurosci Res 45: 161–173.