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Receptor (CD46)- and replication-mediated interleukin-6 induction by measles virus in human astrocytoma cells

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A major source of inflammatory cytokines in the measles virus (MV)-infected brain are astrocytes, which produce a variety of soluble mediators including interferons-α/β (IFN-α/β), interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). Using the MV-strain Edmonston (ED) and the recombinant MV-strain MGV in which the MV-envelope proteins H and F have been replaced by the vesicular stomatitis virus (VSV) envelope protein G, we investigated IL-6 induction in human U-251 astrocytoma cells in the presence and absence of a MV-specific receptor (CD46) interaction. The CD46-MV interaction did not inhibit the induction of cytokines. Similar multiplicities of infection of MGV induced generally lower levels of IL-6 than MV-ED. UV-inactivated replication-incompetent MV-ED induced low levels of IL-6. In contrast, MGV did not induce IL-6 after inactivation with UV light, indicating that the MV-ED-receptor interaction or the uptake of viral particles by membrane fusion induced IL-6, whereas interaction with the VSV-G receptor and uptake of viral particles by endocytosis did not induce IL-6. Crosslink of the MV-receptor CD46 with antibodies and treatment of cells with purified viral glycoproteins led to the induction of small but significant amounts of IL-6. Our data suggest that triggering of CD46 and associated protein kinases can lead to the induction of low levels of IL-6, whereas the replication of the negative strand RNA virus constitutes the major stimulus leading to the synthesis of high levels of IL-6 in astrocytes.

Keywords: recombinant measles virus; measles virus receptor (CD46); astrocytoma cells; cytokine induction; interleukin-6

Introduction

Measles virus (MV) is the etiologic agent inducing the slow virus disease subacute sclerosing panencephalitis (SSPE) in humans (Griffin and Bellini, 1996; Schneider-Schaulies *et al*, 1995c). In such brains, all neural cell types including neurons, astrocytes, oligodendrocytes and microglial cells, as well as epithelial and endothelial cells can be infected with MV (Allen *et al*, 1996; Esolen *et al*, 1995; Kirk *et al*, 1991). In brain regions demonstrating perivascular cell infiltration and gliosis, activated CD4- and CD8-positive T lymphocytes and macrophages were identified within the brain parenchyma. However, the progression of the disease, the viral spread, and the accumulation of characteristic mutations and hypermutations in the viral genome (Baczko *et al*, 1993; Cattaneo *et al*, 1987) are not prevented by the immune response. Both the infiltrating as well as infected neural cells express high levels of inflammatory cytokines (Joncas *et al*, 1976; Hofman *et al*, 1991; McQuaid *et al*, 1997; Nagano *et al*, 1994). Cytokines such as TNF-α and IL-1 are required for the initiation of the immune response, IL-2 and IFN-γ to provide help for effector cells as cytotoxic T cells, and IL-6 for antibody-producing cells. In SSPE brains, the production of high levels of IL-6 is associated with characteristically high levels of oligoclonal anti-MV antibodies (ter Meulen, 1983). Furthermore, IL-6 may in conjunction with other cytokines (IL-1 and IFN-γ) induce gliosis, the local synthesis of neurotropic factors and neuronal degeneration (Campbell *et al*, 1993; Frei *et al*, 1989; Giulian *et al*, 1988; Yong *et al*, 1991).

In vivo and *in vitro* results indicated that a major source for IL-6 are MV-infected astrocytes. In tissue

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culture experiments with rodent and human glial cells it was found that the acute viral infection induces the expression of IL-6, interleukin-1 (IL-1), interferon-α/β (IFN-α/β) and tumor necrosis factor-α (TNF-α) (Lieberman *et al*, 1989; Yamabe *et al*, 1994; Schneider-Schaulies *et al*, 1993). The expression of these cytokines is different when astrocytoma cells were persistently infected with MV showing continual synthesis of lower levels of IL-6 and IFN-β (Schneider-Schaulies *et al*, 1993). These findings suggested that also in the CNS of SSPE patients a percentage of persistently infected astrocytes may continually synthesize IL-6 and IFN-β. Using transgenic mice, it could be demonstrated that the continual intrathecal overexpression of IL-6 *per se* can induce a neurologic disease with neurodegeneration, astrocytosis, angiogenesis and induction of acute-phase-protein production (Campbell *et al*, 1993). These findings indicate that the induction of IL-6 during acute and persistent viral infections of the CNS may have a central pathogenic role in inflammatory neurodegenerative disorders.

It is not well understood how the MV-infection leads to the induction of cytokines such as IL-6 in brain cells and on which level the virus-cell interaction may contribute to triggering signal transduction pathways. In astrocytes, the induction of IL-6 can be achieved by the activation of protein kinase C (PKC) through phorbol myristate acetate (PMA) and calcium ionophores, and is mediated by the transcription factor NF-κB (Libermann and Baltimore, 1990; Norris *et al*, 1994; Palma *et al*, 1995). Recently, it was described that the interaction of MV with its receptor CD46, or a crosslink of CD46 by antibodies has an inhibitory effect on the IL-12 expression by monocytes (Karp *et al*, 1996). In addition, it was found that the cytoplasmic domains of CD46 can interact with multiple protein kinases in macrophages (Wong *et al*, 1997). For other viruses, such as human immunodeficiency virus (HIV) or the respiratory syncytial virus (RSV), it has been described, that the interaction of viral proteins with cell surface receptors or UV-inactivated viruses may be sufficient to induce cytokines (Gessani *et al*, 1997; Stadnyk *et al*, 1997). These findings led us to investigate which steps of the MV-cell interaction and which components of the virus induce the IL-6 expression in the human astrocytoma cells U-251. In addition, with the help of a recombinant MV lacking the MV-glycoproteins (MGV), we analyzed whether the CD46-MV-haemagglutinin interaction may have an inhibitory influence on the induction of IL-6. We investigated the IL-6 expression at three levels: (1) the interaction of MV (strain Edmonston), or antibodies, with the cellular receptor CD46, (2) the binding and uptake of UV-inactivated viral particles including the release of RNPs (ribonucleoprotein particles) in the cytoplasm, and (3) the replication of viral RNA after infection of cells with intact viruses.

Methods

Cells and viruses

The human malignant astrocytoma cell line U-251 (Bigner *et al*, 1981) was cultured in Dulbecco's minimal essential medium (MEM) containing streptomycin (100 μg/ml), penicillin (100 U/ml) and 10% fetal calf serum (FCS), at 37°C and 10% CO_2.

MV strain Edmonston (ED), the recombinant MV named MGV, in which the MV-glycoproteins were substituted by the VSV (vesicular stomatitis virus)-glycoprotein G (Spielhofer *et al*, 1998; Radecke *et al*, 1995; Schlender *et al*, 1996), and VSV strain Indiana were grown on Vero cells. For virus production, cells were infected with a multiplicity of infection (MOI) of 0.01 and virus was harvested when maximum giant cell formation was observed by one cycle of freezing/thawing and two times pelleting cell debris by centrifugation. Supernatants were stored at −80°C. The titres of the viruses were determined by end-point titrations on Vero cells. For a better recognition of infected cells, MGV was stained with antibodies against the MV-nucleocapsid protein and secondary antibodies.

UV-inactivation of viruses

Aliquots of virus (500 μl in 3 cm flasks) were irradiated with 3 J/cm^2 of UV-light (260 nm) using a UV-crosslinker (Bachhofer). The inactivation was assessed by infection of Vero cells with dilutions of irradiated and mock-treated virus. No infectious virus was detected under these conditions in the UV-inactivated virus preparations.

Antibodies, ELISA and inhibitors

The mAbs F227 (anti-MV-N) and 13/42 (anti-CD46 SCR1; Schneider-Schaulies *et al*, 1995a,b) were produced and purified over protein G sepharose in our laboratory. The rabbit polyclonal anti-VSV serum was purchased from Paesel and Lorei GmbH, Germany. The FITC-conjugated rabbit anti-mouse Ig and rabbit anti-human IgG antibodies were purchased from DAKO.

The enzyme linked immunosorbant assay (ELISA) for human IL-6 (IL-6 ELISA Kit, Boehringer Mannheim) was performed as described in the manufacturers manuals. The evaluation of microtiter plates was performed automatically with an ELISA-reader (Biorad) determining the optical density at 492 nm and standardized to samples of cytokines delivered by the manufacturers.

The inhibitors of the protein kinase C (PKC) staurosporin (SS) and of the protein kinase A (PKA) H89 were purchased from Calbiochem. For the analysis of the inhibition of the IL-6 production in the presence of the protein kinase inhibitors, cells were treated with H89 or SS for 30 min prior and during the infection for 1 h with viruses, washed with PBS, and further incubated in the presence of PK-inhibitors.

Glycoprotein preparation
MV preparations were run by ultracentrifugation through a 25% sucrose cushion in NTE-buffer (10 mM Tris pH 7.4, 100 mM NaCl, 1 mM EDTA) at 100 000 × *g* for 90 min at 4°C, and purified virus particles were dissolved in NTE containing 0.3% Triton X-100. The glycoproteins from solubilized viruses were purified by centrifugation on a continuous sucrose gradient (10–40%) in NTE containing 0.3% Triton X-100 at 100 000 *g* for 90 min at 4°C. The top fraction containing the viral glycoproteins was dialyzed against PBS and aliquots were frozen at −70°C.

RNA preparation and Northern blot
U-251 cells were lysed *in situ* by 4 M guanidinium rhodanid buffer and total cellular RNA was pelleted through a caesium chloride cushion as described (Chirgwin *et al*, 1979). Total RNA was separated on 1.5% agarose gels containing 6.3% formaldehyde, blotted on Hybond-N filters (Amersham) and cross-linked with UV light (0.6 Joule/cm^2). The hybridization probe for human IL-6 was the 1.1 kb *Eco*RI fragment of the human IL-6 cDNA (the plasmid was a gift from Dr Capalucci, Genetics Institute, Cambridge, Massachusetts, USA). For control of intact RNA, the 1.4 kb *Pst*I fragment of rat glyceraldhyde 3-phosphate dehydrogenase (GAPDH) cDNA was used. The hybridization probes were radioactively labeled with ^{32}P-dCTP using a random primed labeling kit (Boehringer Mannheim). Blots were exposed to a screen for qualitative and quantitative evaluation with a phosphor imager (Molecular Dynamics).

Flow cytometry
Flow cytometric analyses were performed as described (Schneider-Schaulies *et al*, 1993). Briefly, 2×10^5 cells were fixed 10 min with 3.7% paraformaldehyde containing PBS and then incubated for 45 min on ice with 2 μg of mAb in 200 μl FACS buffer (PBS containing 0.4% BSA and 0.02% sodium azide). Cells were washed twice in FACS buffer and incubated with 200 μl of a 1 : 100 dilution of fluorescein isothiocyanate conjugated goat anti-mouse immunoglobulin (Dako) on ice for a further 45 min. After three washes with FACS buffer, flow cytometric analysis was performed on a FACScan (Becton Dickinson).

Results

Capacity of the human astrocytoma cells U-251 to synthesize IL-6
U-251 cells can synthesize IL-1β, IL-6, IFN-α/β, and TNFα after infection with MV (Schneider-Schaulies *et al*, 1993). Of the inducible cytokines, IL-6 was synthesized to the highest levels, was easily detectable at the RNA and protein level, and was therefore selected as an example to study the induction of cytokines by components of measles virus (MV). In order to determine the maximal level of IL-6 inducible in these cells, we treated the cells either with synthetic dsRNA (Poly I/C) or with phorbol-myristate-acetate (PMA) and calcium ionophore (A23187). Treatment of cells with 100 μg/ml poly I/C led to a IL-6 induction of 6888 pg/10^6 cells after 6 h which decreased at later time points (Table 1). Treatment of cells with PMA (15 nM) and A23187 (0.5 μM) induced maximally 8500 pg/10^6 cells after 48 h (Table 1).

IL-6 induction after infection of human astrocytoma cells with MV-strain ED and recombinant MV MGV
In order to measure the IL-6 levels induced by viral infections in the presence and absence of the receptor binding MV-glycoproteins, we infected the U-251 cells with the MV vaccine strain ED, with the recombinant MV MGV, in which the MV-envelope glycoproteins were substituted by the VSV G protein (Spielhofer *et al*, 1998), and with VSV strain Indiana as a control. ED and MGV induced a dose-dependent synthesis of IL-6, whereas VSV did not induce IL-6 (Figure 1). After 48 h of infection with ED, the low input multiplicity (MOI=0.1) led to an accumulated amount of approximately 750 pg IL-6/10^6 cells in culture, the intermediate multiplicity (MOI=1) led to approximately 1300 pg/10^6 cells, and the high multiplicity led to approximately 1650 pg/10^6 cells (Figure 1, lanes 3, 6 and 9). In comparison, the infection of U-251 cells with MGV induced generally lower levels of IL-6 with approximately 300 pg/10^6 cells at MOI=0.1, 700 pg/10^6 cells at MOI=1 and 1000 pg/10^6 cells at MOI=3 (Figure 1, lanes 12, 15 and 18). Thus, the lack of the MV-glycoproteins was associated with a decreased capacity of the virus to induce IL-6. Infection of cells with VSV or mock infection did not lead to the synthesis of IL-6 above background levels (20–50 pg/10^6 cells; Figure 1, lanes 19–24).

The infection of the U-251 cells was controlled by flow cytometry using a monoclonal antibody (mAb) against the MV nucleocapsid protein or a polyclonal serum against VSV (Figure 2). Figure 2 shows that similar percentages of cells were infected at high MOIs with MV-ED and MGV, although MGV led to a slightly slower infection kinetics as compared to

Table 1 Induction of IL-6 in human astrocytoma cells U-251 by poly I/C, and PMA and calcium ionophore.

Treatment of cells with	*IL-6 in supernatant (pg/10^6 cells)*			
	0 h	*6 h*	*24 h*	*48 h*
poly I/C	40±16	6888±148	4741±113	3558±95
PMA+A23187	40±16	6000±170	7600±285	8500±340

The IL-6 concentration was measured by ELISA with 10^6 U-251 cells in 5 ml medium.

ED. Since VSV rapidly leads to the lysis of U-251 cells, we did not use higher input multiplicities than MOI=1 for VSV. These data indicated that MV-ED can induce considerably higher levels of IL-6 than MGV in U-251 cells, although similar percentages of cells were infected.

In order to analyze whether similar differences are detectable on the mRNA level, we performed Northern blots with RNAs of U-251 cells infected with ED, MGV (Figure 3A,B), and VSV (Figure 3E). VSV Indiana did not induce significant levels of IL-6 mRNA. In the case of infection with ED (MOI=1.0), we found a reproducible characteristic biphasic pattern of the IL-6 mRNA with high levels at 6 and 24 h, and lower levels at 16 and 48 h p.i. (Figure 3A). This pattern is neither detected at low MOIs (MOI=0.1), where the IL-6 mRNA is detected only at later time points, nor at high MOIs (MOI=3 and 5), where signals are stronger at early time points (Schneider-Schaulies *et al*, 1993). After infection of

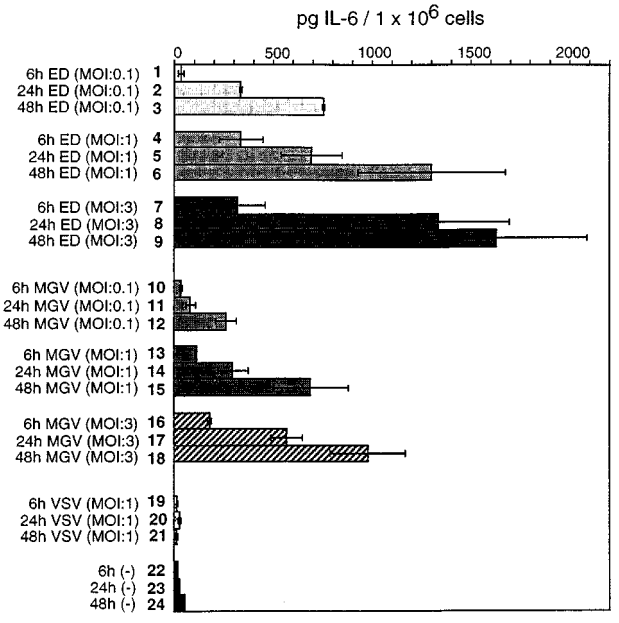

Figure 1 IL-6 in the supernatants of infected U-251 astrocytoma cells. U-251 cells were infected with MV-strain ED (lanes 1–9) and the MV recombinant MGV (lanes 10–18) with MOIs of 0.1, 1 and 3, as indicated, or as control with VSV (Indiana; MOI=1; lanes 19–21), or mock-infected with medium (lanes 22–24). After 6, 24 and 48 h post infection the concentration of IL-6 in the supernatants was measured by ELISA, and is given as pg IL-6 pro 10^6 cells.

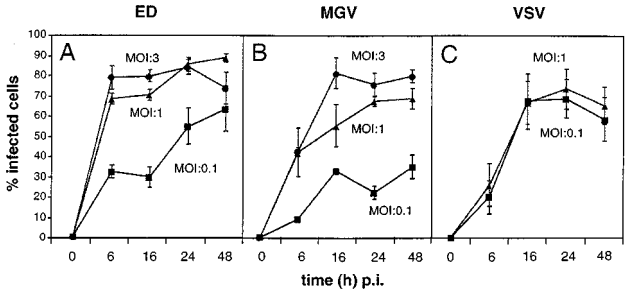

Figure 2 Infection of U-251 astrocytoma cells by MV-ED, MGV and VSV. Cells were infected with various MOIs as indicated, incubated for 6, 16, 24 and 48 h, fixed and stained with monoclonal antibodies against the MV-nucleocapsid protein or with an anti-VSV serum and secondary fluorescein-conjugated antibodies. The percentage of positive cells was determined by flow cytometry.

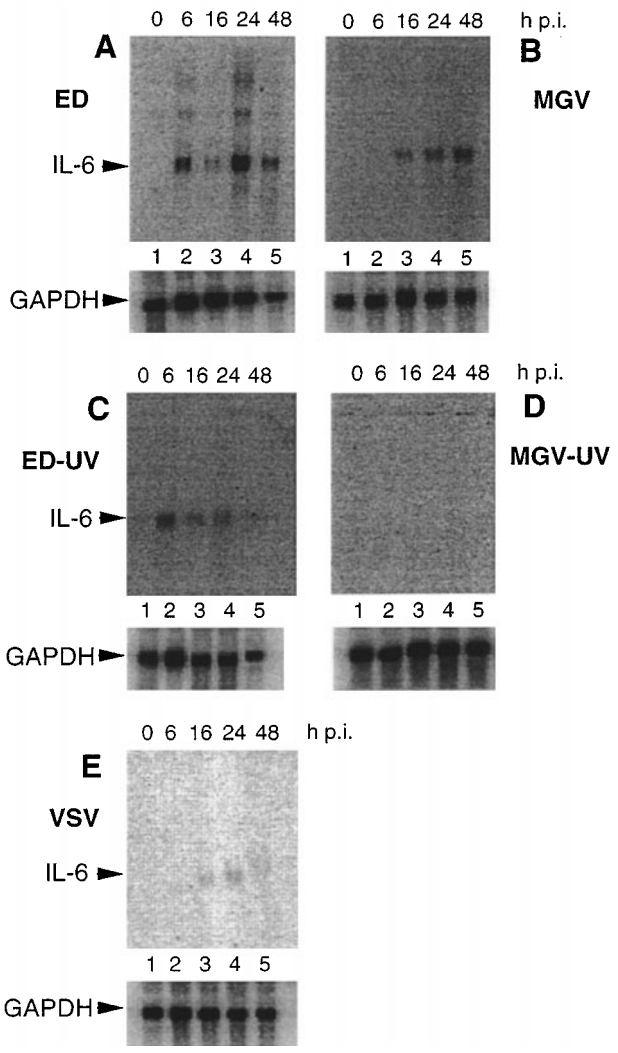

Figure 3 IL-6 mRNA on Northern blots of RNA from U-251 astrocytoma cells. Cells were infected with MV-ED (**A**) and MGV (**B**) with MOIs of 1 for 0, 6, 16, 24 and 48 h. The same amounts of MV-ED and MGV as used for infection were irradiated with UV-light to completely inactivate the infectivity and used for mock-infection of U-251 cells for the same times (**C**, **D**). As control, cells were infected with VSV MOI=1 for the same times. Total cellular RNA was isolated, blotted and hybridized with ^{32}P-labeled specific probes for the IL-6 mRNA and GAPDH as control for the amount of RNA in each lane. The signals were visualized and quantified using a phosphor imager.

U-251 cells with MGV (MOI=1), the IL-6 mRNA was detected after 16, 24 and 48 h p.i. In contrast to ED, higher MOIs of MGV did not induce high levels of IL-6 mRNA at 6 h p.i. Since the difference between MV-ED and MGV is the lack of the MV-glycoproteins F and H in MGV, these data indicated that the presence of the MV glycoproteins is associated with the early IL-6 induction by MV.

Interaction of UV-irradiated MV-particles with U-251 cells induces low levels of IL-6

The glycoproteins F and H of MV-ED are necessary for the proper interaction of virus particles with its cellular receptor CD46 and subsequent pH independent fusion of MV with the cellular membrane. In contrast, MGV infects cells by using a different cellular receptor and a different uptake mechanism via receptor-mediated endocytosis (Mastromarino *et al*, 1987). In order to separate early effects of the interaction of the two viruses with the cell surface (attachment, fusion, and release of nucleocapsids) from viral replication, we used replication-incompetent UV-inactivated MV-ED and MGV. After irradiation of the viruses with 3 J/cm^2, no residual infectious virus particles were detected. The Northern blot analysis of U-251 cells treated with UV-inactivated MV-ED and MGV with an amount of virus corresponding to a MOI of 1 of untreated viruses is shown in Figure 3C and D, respectively. UV-inactivated MV-ED induced a transient IL-6 mRNA synthesis after 6 h with declining levels afterwards. In contrast, IL-6 mRNA could not be detected after treatment of cells with UV-inactivated MGV.

Similar results were observed by measuring IL-6 levels in the cell supernatant (Figure 4). UV-inactivated MV-ED (corresponding to MOIs of 1 and 3) induced 200–300 pg IL-6/10^6 cells after 6 h. This level did not increase at later time points indicating that only the initial interaction between UV-inactivated viruses and the cell led to a transient induction of IL-6 (Figure 4, lanes 4–9). The treatment of cells with UV-inactivated MGV led to a slightly increased IL-6 signal above background levels (Figure 4, lanes 10–18). These data indicated that the early interaction of MV-ED with the cell surface led to the induction of a low but significant level of IL-6 mRNA and protein. This effect could be due to the attachment of the MV glycoproteins with cellular receptors, to the fusion process, or due to the release of viral nucleocapsids into the cytoplasm.

Interaction of the MV envelope glycoproteins F and H with U-251 cells, and crosslink of CD46 by antibodies induce low levels of IL-6

To investigate the possibility that the interaction of MV-ED with cellular receptors is responsible for the induction of the detected low levels of IL-6, U-251 cells were treated with the anti-CD46 mAb 13/42 alone or in the presence of a crosslinking secondary

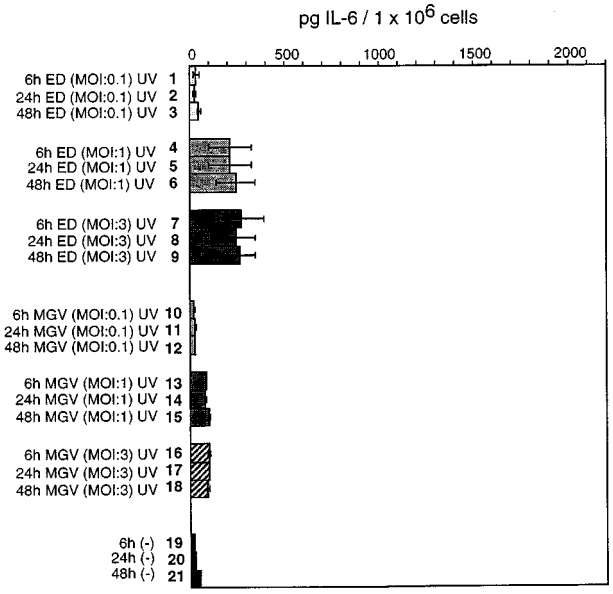

Figure 4 IL-6 in supernatants of U-251 cells treated with UV-inactivated viruses. U-251 cells were treated with the UV-inactivated viruses MV-ED and MGV (as indicated) in amounts corresponding to MOIs of 0.1, 1.0 and 3.0 under similar conditions as in Figure 1. The IL-6 concentration in the supernatants was measured after 6, 24 and 48 h by ELISA. A virus free cell supernatant (virus pelleted by ultracentrifugation) from MV-infected Vero cells was used as mock-infection control (lanes 19–21).

mAb for 6, 24 and 48 h (Figure 5A). The anti-CD46 mAb alone and the secondary antibody alone did not induce IL-6 above background (Figure 5A, lanes 4–6 and 10–12). However, the crosslink of the CD46 antibody with the secondary antibody led to a significant induction of IL-6 after 48 h (Figure 5A, lane 9). With approximately 130 pg/10^6 cells, this induction was not as efficient as treatment of cells with UV-inactivated MV (approximately 250 pg/10^6 cells; Figure 4). This might be due to the much stronger crosslinking activity of the viral surface, or the additional steps of fusion and release of nucleocapsids.

In a further set of experiments we treated the cells with the purified viral glycoproteins H and F. Using the MV-glycoproteins in an amount corresponding to a MOI of 1 of infectious virus as determined by Western blot (not shown), also a low but significant induction of IL-6 could be detected (Figure 5B). These data suggest that CD46 may play a role in the early signal transduction leading to the low level induction of IL-6.

Participation of protein kinase in the induction of IL-6 by MV

To investigate the role of protein kinases for virus induced signal transduction, we used the inhibitors

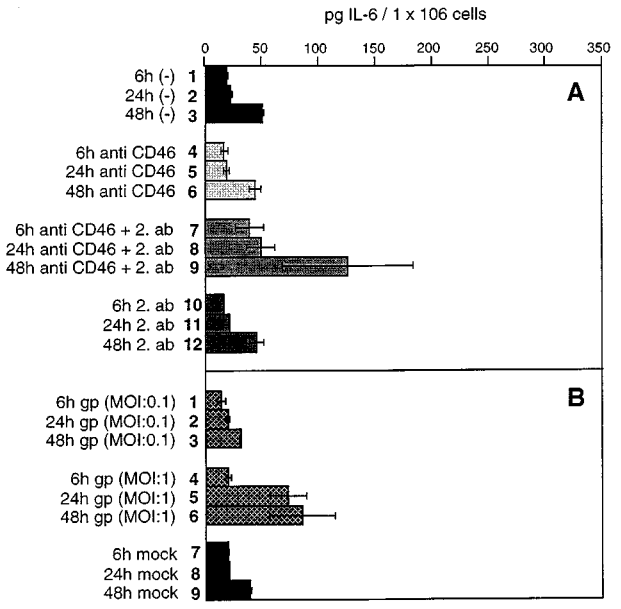

Figure 5 IL-6 induction by crosslink of CD46 and treatment of cells with MV-glycoproteins. (**A**) U-251 cells were incubated without antibody (lanes 1–3), with anti-CD46 mAb 13/42 (100 μg/ml; lanes 4–6), with anti-CD46 and secondary antibodies against mouse IgG (lanes 7–9), and with secondary antibodies alone (lanes 10–12) for 6, 24 and 48 h as indicated. (**B**) U-251 cells were treated with glycoproteins prepared from MV-ED, the protein concentration of which corresponded to MOIs of 0.1 and 1.0, for 6, 24 and 48 h (lanes 1–6). As control, the cells were mock treated with glycoprotein-free buffer (lanes 7–9). The concentration of IL-6 in the supernatants was measured by ELISA.

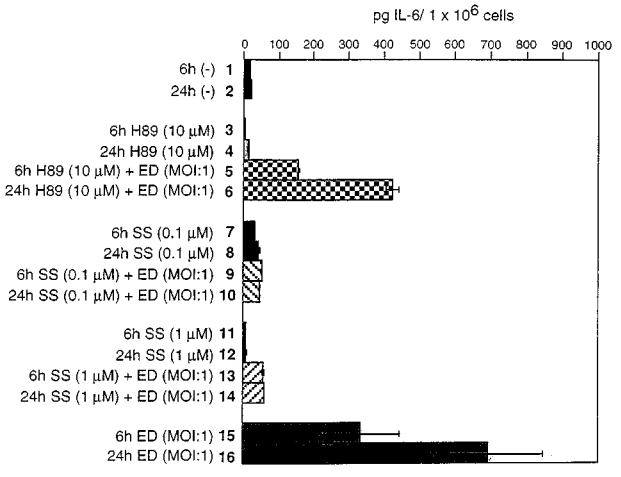

Figure 6 Inhibition of the MV-ED-infection mediated IL-6 induction by protein kinase inhibitors. U-251 cells were treated with Vero cell supernatants (lanes 1, 2), the PKA inhibitor H89 (10 μM, lanes 3, 4), and the PKC inhibitor staurosporin (SS, 0.1 and 1 μM, lanes 7, 8 and 11, 12) in the absence of viral infection as negative controls. Cells were infected with MV-ED after 30 min pretreatment with, and in the presence of the inhibitors H89 (lanes 5 and 6) and staurosporin (0.1 and 1 μM, lanes 9, 10 and 13, 14), or infected with MV-ED without inhibitors as positive controls (lanes 15, 16). The concentration of IL-6 in the supernatants was measured by ELISA.

staurosporin, an inhibitor of protein kinases, mainly PKC, and H89, an inhibitor of PKA. In comparison to 6 and 24 h MV-ED infected U-251 cells (Figure 6, lanes 15 and 16), the IL-6 accumulation was inhibited in the presence of H89 by approximately 50% and by staurosporin by 80–90% after 6 and 24 h (Figure 6). Similar results were observed on the mRNA level. Low concentrations of staurosporin alone (0.1 μM) could induce low levels of IL-6 mRNA (Figure 7A) as described earlier (Palma *et al*, 1995). The MV-ED infection in the presence of 0.1 μM staurosporin led to low levels of IL6 mRNA. Only the mRNA level after 6 h was significantly different from staurosporin treatment alone (factor 2.2 over background in comparison to 1.4 with staurosporin alone, the control in Figure 7B, lane 9 is 10.6 above background, as determined by the phosphorimager). Higher concentrations of staurosporin (1 μM) completely inhibited the induction of IL-6 mRNA (Figure 7B). In the presence of the inhibitor H89, the MV-ED infection led to an IL-6 mRNA signal after 16 h (very weak) and 24 h (factor 2.0 above background; Figure 7C). These signals resembled the IL-6 induction observed after infection of cells with MGV (Figure 3B). The induction of IL-6 mRNA with UV-inactivated MV-ED was completely inhibited by H89 (Figure 7C).

Discussion

In tissue culture, the infection of human glial cells with MV induces the expression of a set of inflammatory cytokines including IL-1, IL-6, IFN-α/β, and TNF-α (Liebermann *et al*, 1989; Schneider-Schaulies *et al*, 1993). For other viral systems it has been shown that the interaction of cells with viral glycoproteins at the cell surface can induce the synthesis of various cytokines. For example in case of the human immunodeficiency virus (HIV) or respiratory syncytial virus (RSV), it has been described that the interaction of viral proteins or UV-inactivated viruses with cell surface receptors may be sufficient to induce cytokines (Gessani *et al*, 1997; Stadnyk *et al*, 1997). In addition, the induction of IFN by viral glycoprotein(s) according to a lectin-like mechanism was found for lymphoid cells (for review see: Ito, 1994). In this system, UV-irradiated Sendai virus (SV), Newcastle Disease virus (NDV), and also influenza virus could induce IFN in mouse spleen cells. The hemolytic and neuraminidase activities of the viruses were not required, and the hemagglutinating activity was sufficient to induce IFN. In contrast to these para- and orthomyxoviruses, the hemagglutinin of MV-ED does not act as a lectin-like molecule, but uses CD46 as cellular receptor (Dörig *et al*, 1993; Manchester *et al*, 1994; Naniche *et al*, 1993). Recently, Karp *et al*, described that the IL-12 production in monocytes and macrophages was

specifically inhibited by MV-infection, as well as after interaction of CD46 with the complement factor C3b, or crosslinking of CD46 by antibodies.

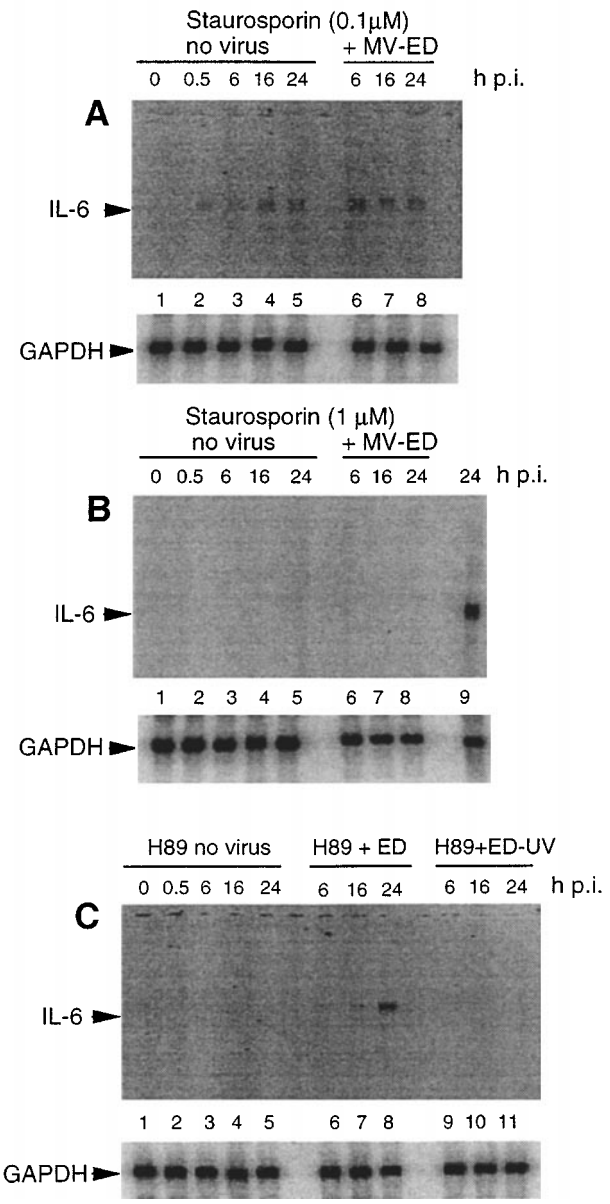

Figure 7 IL-6 mRNA on Northern blots of RNA from U-251 cells treated with protein kinase inhibitors. U-251 cells were treated with 0.1 μM (**A**) and 1.0 μM (**B**) staurosporin either in the absence of virus as negative control (lanes 1–5), or 30 min before, during and after infection of cells MV-ED (MOI=1) for 0.5, 6, 16 and 24 h as indicated (lanes 6–8). In (**B**) an additional lane (lane 9) with RNA from 24 h MV-ED infected U-251 cells without inhibitor was used as positive control for the hybridization with the IL-6 probe. In (**C**), U-251 cells were treated with the PKA inhibitor H89 alone (lanes 1–5), treated with H89 and infected with MV-ED (lanes 6–8), and treated with H89 and with UV-inactivated MV-ED (MOI corresponding to 1.0, lanes 9–11). Total cellular RNA was isolated, blotted and hybridized with a ^{32}P-labeled IL-6 mRNA specific probe. The signals were visualized and quantified using a phosphor imager.

The IL-6 induction was not negatively affected in these cells (Karp *et al*, 1996). These findings strongly suggested that the induction of a signal transduction cascade takes place through the MV-receptor CD46.

In order to investigate a possible involvement of CD46 in the inhibition or induction of cytokines in human astrocytoma cells (U-251), we analyzed the expression of the cytokine IL-6, which is easily detected in SSPE brains and *in vitro* after MV-infection. We found that the interaction of UV-inactivated MV-ED with the cells, the interaction of purified viral glycoproteins with the cells, and the crosslink of CD46 with antibodies, induced small amounts of IL-6. In contrast, high levels of IL-6 were induced after infection of cells with replication competent MV. Using the recombinant measles virus MGV containing the VSV-G protein instead of the MV-envelope proteins H and F, we found no induction of IL-6 by UV-inactivated virus. After infection with replication-competent MGV, IL-6 was strongly induced, however to lower levels than after infection with MV-ED. Thus, absence of the MV-glycoproteins was associated with a lower virus-induced IL-6 production. It was clearly not associated with a greater capacity to induce IL-6, which could have been the case if the MV-glycoproteins had exerted an inhibitory effect on the IL-6 induction in these cells. The biphasic induction of IL-6 after infection with MV-ED can be interpreted according to the occurrence of reinfection in tissue culture by newly synthesized viruses after 24 h. The IL-6 synthesis after 6 h of infection was stronger with replication competent ED than using UV-inactivated virus, presumably because of the immediate start of transcription and the emergence of dsRNA intermediates. We cannot exclude that the observed low induction of IL-6 after virus-cell surface interaction is only a side effect of an efficient signalling cascade acting on unknown functions of the cells. Since crosslink of CD46 by antibodies led to a weaker IL-6 induction than treatment of cells with UV-inactivated MV-ED, it is also possible that unknown receptors in addition to CD46 may play a role in the virus-induced signal transduction.

In the presence of an inhibitor of PKA (H89), UV-inactivated MV-ED did not induce IL-6 after 6 h. Thus, the receptor-mediated induction of low amounts of IL-6 may require the action of this kinase. In comparison, after infection of cells with replication-competent MV-ED for 6–48 h, H89 reduced the IL-6 mRNA and protein synthesis by approximately 50% and the PKC inhibitor staurosporin by 80–90%. H89 completely inhibited the induction of IL-6 by UV-inactivated MV-ED. Our results support the findings of Wong *et al*, 1997 that kinases are associated with the cytoplasmic domain of CD46. However, since these substances are relatively unspecific inhibitors of several kinases

and inhibit many cell functions including proliferation and viral replication, we cannot conclude whether the observed effects on the IL-6 synthesis are direct or rather indirect. Nevertheless, the results show that the action of these kinases is a prerequisite for the induction of IL-6 either via receptors or intracellularly. These data suggest that possibly also *in vivo* already the surface interaction of (inactivated) MV or infected cells with receptor bearing cells may induce the synthesis of low levels of the inflammatory cytokine IL-6, and that induction of high levels of IL-6 requires the active replication of MV.

The strong induction of IL-6 by actively replicating MV may be due to the presence of double stranded RNA-intermediates in the cytoplasm of infected cells. In the astrocytoma cells MV-replication induced up to 1700 pg/ml IL-6, whereas the addition of artificial double stranded RNA (poly IC) induced approximately 7000 pg/m IL-6 and PMA plus A23187 induced even higher amounts of IL-6 up to 8500 pg/ml (Table 1). Thus, also the infection of cells with a high MOI of MV-ED could not induce the full capacity of the U-251 cells to produce IL-6, and may reflect the limited accessibility of double-stranded RNA intermediates during the replication of negative-strand RNA viruses. In comparison to such high levels of IL-6, it is not clear whether the low levels induced by the virus-CD46 interaction are of any significance. A possible reason for the observed differences after infection with replication-competent MGV and MV-ED may be the usage of different receptors for the entry of these viruses and the subsequent localization in intracellular compartments. Determined by the VSV-G protein, MGV is taken up by receptor-mediated endocytosis in acidic endosomes (Spielhofer *et al*, 1998), a pathway which may not be ideal for the MV-specific replication. This may be reflected by the slightly lower synthesis of viral proteins (Figure 2) and by the generally lower level of IL-6 detected after infection of the cells with same MOIs as used for MV-ED (Figure 1).

What conclusions can be drawn for the *in vivo* situation? In tissue culture, the level of IL-6 synthesis correlates with the level of MV-replication. In persistently infected cells, which produce less virus, the extent of the IL-6 synthesis is drastically reduced (Schneider-Schaulies *et al*, 1993). It is likely that also in MV-infected brains the level of the IL-6 correlates with the extent of viral replication. In comparison to the high amounts of IL-6 synthesized in response to replication, the small amounts of IL-6 induced by MV-CD46 interaction may play only a marginal role. This suggestion is supported by the findings in SSPE brains, where usually very little virus-specific glycoprotein is detected due to the restriction of the viral glycoprotein expression by antiviral antibodies and the steep transcriptional gradient in neural cells (Cattaneo *et al*, 1987; Liebert *et al*, 1990; Schneider-Schaulies *et al*, 1992). In addition, only little CD46 is expressed by neurons and astrocytes in the human brain in comparison to levels of CD46 on endothelial cells or lymphocytes (Gordon *et al*, 1992; Ogata *et al*, 1997). Therefore, we suggest that in the infected brain the intracellular viral replication is the main mechanism regulating the induction and the level of IL-6.

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References

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

Baczko K, Lampe J, Liebert UG, Brinckmann U, ter Meulen V, Pardowitz J, Budka H, Cosby SL, Isserte S, Rima BK (1993). Clonal expansion of hypermutated measles virus in a SSPE brain. *Virology* **197:** 188–195.

Bigner DD, Bigner SH, Ponten J, Westermark B, Mahaley MS, Rouslahti E, Herschman H, Eng LF, Wikstrand CJ (1981). Heterogeneity of genotypic and phenotypic characteristics of fifteen permanent cell lines derived from human gliomas. *J Neuropathol Exp Neurol* **40:** 201–229.

Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MBA, Mucke L (1993). Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin-6. *Proc Natl Acad Sci USA* **90:** 10061–10065.

Cattaneo R, Rebmann G, Schmid A, Baczko K, ter Meulen V, Billeter MA (1987). Altered transcription of a defective measles virus genome derived from a diseased human brain. *EMBO J* **6:** 681–687.

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ (1979). Isolation of biologically active ribonucleic acids from sources enriched in ribonuclease. *Biochemistry* **18:** 5294–5299.

Dörig RE, Marcil A, Chopra A, Richardson CD (1993). The human CD46 molecule is a receptor for measles virus (Edmontson strain). *Cell* **75:** 295–305.

Esolen LM, Takahashi K, Johnson RT, Vaisberg A, Moench TR, Wesselingh SL, Griffin DE (1995). Brain endothelial cell infection in children with acute fatal measles. *J Clin Invest* **96:** 2478–2481.

Frei K, Malipiero JV, Leist TP, Zinkernagel RM, Schwab ME, Fontana A (1989). On the cellular source and function of interleukin-6 produced in the central nervous system in viral disease. *Eur J Immunol* **19:** 689–694.

Gessani S, Borghi P, Fantuzzi L, Varano B, Conti L, Puddu P, Belardelli F (1997). Induction of cytokines by HIV-1 and its gp120 protein in human peripheral blood monocyte/macrophages and modulation of cytokine response during differentiation. *J Leukoc Biol* **62:** 49–53.

Giulian D, Woodward J, Young DG, Krebs JF, Lachman LB (1988). Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J Neurosci* **8:** 2485–2490.

Gordon DL, Dadlon TA, Wesselingh SL, Russell SM, Johnstone RW, Purcell DFJ (1992). Human astrocytes express membrane cofactor protein (CD46), a regulator of complement activation. *J Neuroimmunol* **36:** 199–208.

Griffin DE, Bellini WJ (1996). Measles Virus. In: *Fiels Virology*. Fields BN, Knipe DM, Howley PM *et al*. (Eds). Lippincott-Raven Publishers, Philadelphia, pp. 1267–1312.

Hofman FM, Hinton DR, Baemayr J, Weil M, Merrill JE (1991). Lymphokines and immunoregulatory molecules in subacute sclerosing panencephalitis. *Clin Immunol Immunopathol* **58:** 331–342.

Ito Y (1994). Induction of interferon by virus glycoprotein(s) in lymphoid cells through interaction with the cellular receptors via lectin-like action: An alternative interferon induction mechanism. *Arch Virol* **138:** 187–198.

Joncas JH, Robillard LR, Boudreault A, Leyritz M, McLaughlin BJM (1976). Interferon in serum and cerebrospinal fluid in subacute sclerosing panencephalitis. *Can Med Assoc J* **115:** 309–313.

Karp CL, Wysocka M, Wahl LM, Ahearn JM, Cuomo PJ, Sherry B, Trinchieri G, Griffin DE (1996). Mechanism of Suppression of cell-mediated immunity by measles virus. *Science* **273:** 228–231.

Kirk J, Zhou AL, McQuaid S, Cosby SL, Allen IV (1991). Cerebral endothelial cell infection by measles virus in subacute sclerosing panencephalitis: ultrastructural and in situ hybrididdation evidence. *Neuropathol Appl Neurobiol* **17:** 289–297.

Libermann TA, Baltimore D (1990). Activation of interleukin-6 gene expression through the NF-κB transcription factor. *Mol Cell Biol* **10:** 2327–2334.

Lieberman AP, Pitha PM, Shin HS, Shin ML (1989). Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. *Proc Natl Acad Sci USA* **86:** 6348–6352.

Liebert UG, Schneider-Schaulies S, Baczko K, ter Meulen V (1990). Antibody-induced restriction of viral gene expression in measles encephalitis in rats. *J Virol* **64:** 706–713.

Manchester M, Liszewski MK, Atkinson JP, Oldstone MBA (1994). Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus. *Proc Natl Acad Sci USA* **91:** 2161–2165.

Mastromarino P, Conti C, Goldoni P, Hauttecoer B, Orsi N (1987). Characterization of membrane components of the erythrocyte involved in vesicular stomatitis virus attachment and fusion at acidic pH. *J Gen Virol* **68:** 2359–2369.

McQuaid S, Campbell R, Isserte S, Cosby SL (1997). Leukemia inhibitory factor mRNA is expressed in the brains of patients with subacute sclerosing panencephalitis. *J Neuroimmunol* **77:** 57–62.

Nagano I, Nakamura S, Yoshioka M, Onodera J, Kogure K, Itoyama Y (1994). Expression of cytokines in brain lesions in subacute sclerosing panencephalitis. *Neurology* **44:** 710–715.

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.

Norris JG, Tang LP, Sparacio SM, Benveniste EN (1994). Signal transduction pathways mediating astrocyte IL-6 induction by IL-1 beta and tumor necrosis factor-alpha. *J Immunol* **152:** 841–850.

Ogata A, Czub S, Ogata S, Cosby SL, McQuaid S, Budka H, ter Meulen V, Schneider-Schaulies J (1997). Absence of measles virus receptor (CD46) in lesions of subacute sclerosing panencephalitis brains. *Acta Neuropathol* **94:** 444–449.

Palma C, Urbani F, Manzini S (1995). Interleukin-6 production by U373 MG, a human astrocytoma cell line: different pathways involved in substance P and lipopolysaccharide activation. *J Neuroimmunol* **59:** 155–163.

Radecke F, Spielhofer P, Schneider H, Kaelin K, Huber M, Dötsch C, Christiansen G, Billeter M (1995). Rescue of measles virus from cloned DNA. *The EMBO J* **14:** 5773–5784.

Schlender J, Schnorr J-J, Spielhofer P, Cathomen T, Cattaneo R, Billeter MA, ter Meulen V, Schneider-Schaulies S (1996). Interaction of measles virus glycoproteins with the surface of uninfected peripheral blood lymphocytes induces immunosuppression in vitro. *Proc Natl Acad Sci USA* **93:** 13194–13199.

Schneider-Schaulies J, Dunster LM, Schwartz-Albiez R, Krohne G, ter Meulen V (1995a). Physical association of moesin and CD46 as a receptor complex for measles virus. *J Virol* **69:** 2248–2256.

Schneider-Schaulies J, Schneider-Schaulies S, ter Meulen V (1993). Differential induction of cytokines by primary and persistent measles virus infections in human glial cells. *Virology* **195:** 219–228.

Schneider-Schaulies J, Schnorr J-J, Brinckmann U, Dunster LM, Baczko K, Schneider-Schaulies S, ter Meulen V (1995b). Receptor usage and differential downregulation of CD46 by measles virus wild type and vaccine strains. *Proc Natl Acad Sci USA* **92:** 3943–3947.

Schneider-Schaulies S, Liebert UG, Segev Y, Rager-Zisman B, Wolfson M, ter Meulen V (1992). Antibody-dependent transcriptional regulation of measles virus in persistently infected neural cells. *J Virol* **66:** 5534–5541.

Schneider-Schaulies S, Schneider-Schaulies J, Dunster LM, ter Meulen V (1995c). Measles virus gene expression in neural cells. In: *Current Topics of Microbiology and Immunology (CTMI)* Ed. Billeter and ter Meulen. **191:** 101–116.

Spielhofer P, Bächi T, Fehr T, Christiansen G, Cattaneo R, Kaelin K, Billeter MA, Naim HY (1998). Chimeric measles virus with a foreign envelope. *J Virol* **72:** 2150–2159.

Stadnyk AW, Gillan TL, Anderson R (1997). Respiratory syncytial virus triggers synthesis of IL-6 in BALB/c mouse alveolar macrophages in the absence of viral replication. *Cell Immunol* **176:** 122–126.

ter Meulen V, Stephenson JR, Kreth HW (1983). Subacute sclerosing panencephalitis. *Comprehensive Virology* **18:** 105–159.

Wong TC, Yant S, Harder BJ, Korte-Sarfaty J, Hirano A (1997). The cytoplasmic domains of complement regulatory protein CD46 interact with multiple kinases in macrophages. *J Leukoc Biol* **62:** 892–900.

Yamabe T, Dhir G, Cowan EP, Wolf AL, Bergey GK, Krumhoz A, Barry E, Hoffman PM, Dhib-Jalbut S (1994). Cytokine-gene expression in measles-infected adult human glial cells. *J Neuroimmunol* **49:** 171–179.

Yong VW, Moumdjian R, Yong FP, Ruijs TC, Freedman MS, Cashman N, Antel JP (1991). Gamma-interferon promotes proliferation of adult human astrocytes in vitro and reactive gliosis in the adult mouse brain in vivo. *Proc Natl Acad Sci USA* **88:** 7016–7020.