Reversal of the measles virus-mediated increase of phosphorylating activity in persistently infected mouse neuroblastoma cells by anti-measles virus antibodies

Y. Segev, B. Rager-Zisman, N. Isakov, S. Schneider-Schaulies, V. ter Meulen, S. Udem, S. SegaP and M. Wolfson*

1*Ben Gurion University of the Negev, Department of Microbiology and Immunology, P.O. Box 653, 84105 Beer Sheva, Israel, 2Institut für Virologie und Immunbiologie, Universität Würzburg, Versbacher Strasse 7, 97078 Würzburg, Germany and 3New Jersey Medical School-UMDNJ, Department of Medicine, Newark, New Jersey 07163, U.S.A.

To investigate the effect of persistent measles virus infection on signal transduction in cells of neuronal origin, the mouse neuroblastoma cell line NS20Y/MS, which is persistently infected with measles virus, was used. The results demonstrate an approximate 50% increase in total phosphorylation and a similar increase in protein kinase C (PKC) activity. Western blot analysis with anti-total PKC or anti-PKC-α antibodies revealed a significant increase in the level of an SOK immuno-reactive PKC in NS20Y/MS cells. Following incubation of NS20Y/MS cells with polyclonal anti-measles virus antibodies, which down-regulate the level of measles virus proteins, total and PKC-mediated phosphorylation returned to the basal level of uninfected cells. This effect was reversible and removal of the antibodies resulted in restoration of the high level of total and PKC-mediated phosphorylation. The release of infectious measles virus was strongly inhibited by incubation of NS20Y/MS cells with the PKC inhibitor, 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H-7). These results demonstrate that measles virus induces elevation in cellular phosphorylation which is essential for measles virus production.

Introduction

Measles virus (MV), a paramyxovirus, is highly contagious and is one of the major causes of infant mortality in underdeveloped countries (Bloom, 1989). Sporadic outbreaks of acute measles still occur in industrialized countries despite vaccination (Centers for Disease Control, 1989) and a recent epidemic has caused more than 100 deaths in North America. MV can cause four distinct syndromes in humans: the classical epidemic acute illness of children, an autoimmune-like condition known as post-infectious encephalomyelitis (Norby & Oxman, 1990; Kipps et al., 1983), late and usually fatal complications of acute measles infection known as subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis (MIBE). These complications develop as a result of the persistence of measles virus in brain cells (Baczko et al., 1984; Basle et al., 1987; Carter et al., 1983; Cattaneo et al., 1987, 1988; Kipps et al., 1983; Payne et al., 1969). MV replication in the brains of SSPE and MIBE patients is characterized by defective expression of the viral envelope proteins (Baczko et al., 1984, 1988; Cattaneo et al., 1988). In these cases the virus persists despite the presence of high titres of antiviral antibodies and competent immune cells.

Protein phosphorylation is a common mechanism for the regulation of physiological functions of cells. Many key regulatory proteins exist in cells in either a phosphorylated or a dephosphorylated form, their steady-state levels of phosphorylation reflecting the relative activities of the protein kinases and protein phosphatases that catalyse the interconversion processes. These kinases and phosphatases are often targets themselves for regulation by protein phosphorylation as part of a cascade in signal transduction pathways. The basic mechanism for signal transduction is that the extracellular signal affects the activity of a protein kinase cascade that modulates transcription factor activity by phosphorylation and results in alterations in cellular gene expression (Karin, 1991, 1992; Nigg, 1990; Schwartz & Greenberg, 1987).

One signal transduction pathway that is of special importance in the regulation of brain cell functions uses...
phosphatidylinositol 4,5-bisphosphate (PIP2) as a precursor molecule for two second messengers. Ligand binding by receptors that operate via this pathway results in activation of a phospholipase C that hydrolyses PIP2 to yield diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). DAG activates protein kinase C (PKC) which leads to phosphorylation of numerous endogenous substrates, and IP3 releases Ca2+ from intracellular stores and activates various Ca2+-dependent enzymes (Berridge, 1987). PKC is a ubiquitous calcium/lipid-dependent enzyme which phosphorylates a variety of membrane, cytosolic and nuclear proteins on serine/threonine residues (for reviews see Nishizuka, 1986a,b, 1988, 1992). PKC exists as a family of at least eight structurally and functionally related isoenzymes with minor differences in enzymatic properties, substrate specificity and tissue distribution (Kikkawa et al., 1987; Kuo et al., 1986; Huang et al., 1986, 1988; Huang, 1989; Ono et al., 1988; Akita et al., 1990; Sekiguchi et al., 1987; Coussens et al., 1986). Physiologically, PKC is activated by endogenous DAG in response to occupation of membrane receptors by the appropriate ligand (Nishizuka, 1986a; Huang, 1989). An enzymatically active PKC binds tightly to membranes (Kikkawa et al., 1982). This phenomenon, known as translocation, designates activation of the enzyme.

Taking into account the principal role of phosphorylation processes and PKC activity in neuronal functions, one may suggest that any alteration in this enzyme's activity will result in impairment of the normal function of these cells. Indeed, changes in PKC activity have been observed in various patho-physiological conditions. For instance, in short- or long-term forebrain ischemia, translocation of PKC from cytosol to membranes and its subsequent downregulation have been observed (Louis et al., 1988, 1991; Wieloch et al., 1991). Enhanced PKC activity and alterations in its distribution, as well as phosphorylation of a neuron-specific PKC substrate, were found in the neocortex of patients at early stages of Alzheimer's disease (Clark et al., 1991; Florez et al., 1991; Masliah et al., 1991). Several studies have indicated that viral infection may indeed affect phosphoinositide (PI) turnover and the phosphorylating activity of PKC. Mouse fibroblasts infected with herpes simplex virus type 2 exhibited elevated PKC activity (Roddick et al., 1988), whereas mouse fibroblasts infected with human T cell leukemia virus or adenovirus exhibited an increased turnover of PI (Nevins, 1989; Tan et al., 1989).

To investigate the effects of persistent MV infection on cells of the central nervous system we have used a mouse neuroblastoma cell line persistently infected with MV, NS20Y/MS (Rager-Zisman et al., 1984). This cell line was established from clone NS20Y of the C1300 murine neuroblastoma which is capable of differentiating in vitro and retains neuron-specific enzymes and receptor markers (Augusti-Tocco & Sato, 1969; Oldstone et al., 1977). Recent studies by Schneider-Schaulies et al. (1992) have also demonstrated that the restriction of MV gene expression in these cells is similar to that found in brain cells of SSPE patients and experimentally infected rats. Our previous studies demonstrated that NS20Y/MS cells exhibit elevated PKC activity and augmented expression of the c-fos proto-oncogene (Wolfson et al., 1991).

The present study was undertaken to investigate the regulation of protein phosphorylation and PKC activity in NS20Y/MS cells. We demonstrate that the enhanced total cellular phosphorylation and PKC activity could be downregulated by treatment of the persistently infected cells with polyclonal anti-MV antibodies. Following treatment of infected cells with these antibodies, but not with monoclonal antibodies, total and PKC-mediated protein phosphorylation returned to the basal levels of uninfected neuroblastoma cells (NS20Y). In addition we have shown that inhibition of PKC activity by 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H-7) decreased the release of infectious virus from these cells. These results suggest that the enhanced phosphorylating activity of persistently infected cells is induced by the virus and that PKC-mediated phosphorylation plays an important role in the maturation of infectious MV particles.

**Methods**

**Cells and viruses.** The C1300 neuroblastoma, clone NS20Y, of the A/J mouse strain was used. The establishment of MV persistently infected NS20Y cells termed NS20Y/MS is described in detail elsewhere (Rager-Zisman et al., 1984). Cells were routinely grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), glutamine and antibiotics.

**Antibodies.** Polyclonal anti-MV hyperimmune serum was raised in rabbits by immunization with antigen in complete Freund's adjuvant, followed by repeated intramuscular injections with solubilized virus alone (Ja et al., 1978). The preparation and characterization of monoclonal antibodies against measles virus N protein were described by Birrer et al. (1981) and against H protein (NC32) by Carter et al. (1983). Polyclonal rabbit anti-PKC antibodies were prepared as described previously (Isakov et al., 1990). Monoclonal anti-bovine spleen PKCa antibodies were purchased from Seikagaku.

**Measurement of total phosphorylation and PKC activity.** Total phosphorylation and PKC activity were determined in cytosolic and particulate fractions. Cytosolic fractions were obtained by the following procedure. Cells were resuspended in an extraction buffer containing 20 mM-Tris-HCl pH 7.5, 1 mM-PMSEF, 2 mM-EGTA and 10 mM-DTT, disrupted by sonication on ice and centrifuged for 30 min at 20000 g. The supernatant was designated the cytosolic fraction. To obtain membrane-associated proteins, corresponding pellets were extracted for 1 h on ice in the same buffer containing 1% Triton X-100. After centrifugation at 20000 g for 30 min the resulting supernatant was designated the particulate fraction. Both fractions were further purified...
by chromatography on DE52 cellulose columns (Whatman) (Thomas et al., 1987). Histone H1 was used as an exogenous substrate, [γ-32P]ATP (NEC, sp. act. 10 to 50 Ci/mmol) as the phosphate group donor and DAG, phosphatidylserine (PS) and CaCl2, as PKC activators (Kikawa et al., 1982; Wolfson et al., 1985). The standard assay mixture (0.2 ml) contained 20 mM-Tris–HCl pH 7.5, 10 mM-DDTT, 0.4 mM-EGTA, 10 mM-MgCl2, 1 mM-CaCl2, 2 μg/ml DAG and 80 μg/ml PS, histone H1 (30 μg/ml) and [γ-32P]ATP (2 × 106 c.p.m./assay, in a final concentration of 50 μM). Where indicated, 50 mM-phorbol 12-myristate 13-acetate was used instead of DAG. Optimal concentrations of the activators were determined in preliminary experiments. After 3 min incubation at 30°C, the reaction was stopped by the addition of 25% TCA and the reaction mixture was filtered through nitrocellulose filters (0.45 μm) in a dot blot apparatus. The nitrocellulose filter was washed three times with cold 10% TCA, dried, cut and 32P incorporation was measured by liquid scintillation counting. Assays were performed in duplicate in the presence or absence of calcium and lipids. Enzymatic activity was measured as the incorporation of 32P from [γ-32P]ATP into histone H1. Activity is expressed as picomoles of 32P incorporated per min per mg of sample protein. Total phosphorylating activity was calculated in the same units in the absence of PKC cofactors (calcium or lipids). Net PKC activity was calculated by subtracting the activity measured in the absence of the cofactors from that measured in the presence of cofactors. Calculation of the cytosol: particulate ratio is used to demonstrate enzyme translocation.

Endogenous protein phosphorylation in vitro. For analysis of total protein phosphorylation, cell suspensions were washed twice with PBS, resuspended in extraction buffer, disrupted by sonication on ice and centrifuged for 30 min. Reactions were initiated by the addition of [γ-32P]ATP as the phosphate group donor (2 × 106 c.p.m./assay, in a final concentration of 50 mM), for 3 min at 30°C. The reaction was terminated by boiling for 5 min in 2× SDS sample buffer as described by Laemmli (1970). Phosphorylated samples containing 150 to 200 μg protein were then separated by 10% SDS-PAGE for 18 h. Gels were dried and the phosphorylated proteins were detected by autoradiography.

For analysis of PKC-mediated phosphorylation, the same reaction mixture was used with the addition of 2 μg/ml DAG, 80 μg/ml PS and 1 mM-CaCl2 as PKC cofactors.

Western blotting. For protein analysis cell extracts were prepared in cold RIPA buffer (50 mM-Tris–HCl pH 7.5, 0.25% w/v sodium deoxycholate, 0.1% w/v SDS and 0.5% w/v NP40). Extracts were mixed with 2× sample buffer and boiled for 5 min. Then 150 to 200 μg portions of sample protein were loaded in each gel lane and subjected to 10% SDS-PAGE. The resolved proteins were transferred electrophoretically to nitrocellulose filters which were then blocked for 1 h in PBS containing 10% low fat dry milk, followed by 1 h incubation with the first antibody diluted in PBS containing 1% dry milk. The filters were washed three times in PBS and the immunoblots were incubated with peroxidase-conjugated second antibody for 1 h, washed three times with PBS and developed with 4-chloronaphthol (0.3 mg/ml) and H2O2.

Treatment of cells with anti-MV antibodies. For antibody treatment, the medium of semiconfluent cultures was replaced with fresh medium containing anti-MV polyclonal or monoclonal antibodies sufficient to neutralize 104 pfu of MV. Every third day, the cells were split and fresh medium containing antibodies was added. Antibody-treated or untreated cells were harvested at different times after antibody treatment. For reactivation, the cells were cultured in medium without antibodies and harvested at different times after antibody removal.

Treatment of cells with H-7. To study its effect on virus release, H-7 (dissolved in DMSO) was added to confluent cell cultures, 24 h after subculture, at a final concentration of 100 μM. Twenty-four hours later, supernatants were collected and used for titration of the virus. The solvent (DMSO) had no effect on the virus release at the concentration used in the experiments.

Virus titration. The titres of virus in culture supernatants were determined by a semiquantitative plaque assay (Rager-Zisman et al., 1984). Vero cell monolayers were tryptized and plated in 24-well plastic trays. Cells (3 × 105) were seeded in each well and incubated at 37°C overnight. Tenfold dilutions of virus samples were prepared in Dulbecco's MEM supplemented with 2% FCS. The growth medium from each well was aspirated, and duplicate wells were inoculated with 0.2 ml of viral dilutions. After an adsorption period of 2 h at 33°C, 1 ml of growth medium containing 0.75% carboxymethyl cellulose was added. The monolayers were then incubated at 37°C for 5 days, fixed with 10% formol–saline and stained with crystal violet.

Results

In vitro phosphorylation of endogenous proteins in NS20Y/MS cells

The effect of MV persistent infection on total protein phosphorylation was investigated by measuring in vitro incorporation of 32P into proteins as described in Methods. The results demonstrate a 50% increase in total phosphorylating activity in the cytosolic fraction of infected cells NS20Y/MS, as compared with uninfected NS20Y (Fig. 1a). Separation of the phosphorylated proteins by SDS–PAGE showed an overall increase in phosphorylation of endogenous protein substrates in NS20Y/MS cells (Fig. 1b).

Fig. 1. Total protein phosphorylation activity in NS20Y and NS20Y/MS cells. (a) Phosphorylation of histone H1 by cytosolic extracts of NS20Y or NS20Y/MS cells. (b) In vitro endogenous protein phosphorylation of crude cytosolic extracts incubated with [γ-32P]ATP for 3 min, followed by SDS-PAGE. Gels were dried and phosphorylated proteins were visualized by autoradiography. Lane 1, NS20Y; lane 2, NS20Y/MS. Results were representative of at least six experiments.
Table 1. PKC activity in NS20Y/MS and NS20Y cells*

<table>
<thead>
<tr>
<th>Cell</th>
<th>Fraction</th>
<th>TPA</th>
<th>DAG</th>
<th>Increase (%)</th>
<th>Cytosolic: particulate</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS20Y</td>
<td>Cytosol</td>
<td>364±11</td>
<td>329±34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS20Y/MS</td>
<td>Cytosol</td>
<td>564±27</td>
<td>523±31</td>
<td>37%</td>
<td>1·28</td>
</tr>
<tr>
<td>NS20Y</td>
<td>Particulate</td>
<td>278±18</td>
<td>256±43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS20Y/MS</td>
<td>Particulate</td>
<td>426±17</td>
<td>396±25</td>
<td>36%</td>
<td>1·32</td>
</tr>
</tbody>
</table>

* Endogenous activity of PKC (pmol/min/mg protein) was measured in the cytosolic and particulate extracts of NS20Y and NS20Y/MS cells as described in Methods. TPA (50 nm) or DAG (2 μg/ml) were added to the reaction mixture and PKC activity was determined on 50 mg protein samples. Results are the mean of three experiments±s.D.

Protein kinase C-mediated activity and phosphorylation in NS20Y/MS cells

To examine the extent of phosphorylation mediated by PKC, we assayed the activity of these enzymes using either DAG with PS or TPA with PS as specific PKC cofactors. The results summarized in Table 1 demonstrate an increased PKC activity in both fractions of NS20Y/MS cells being 37% and 36% in cytosolic and particulate fractions, respectively. The ratio of cytosolic:particulate PKC activity remained practically unchanged, 1·32 in NS20Y/MS cells and 1·28 in uninfected NS20Y cells. These results indicate that the accumulation of viral proteins in NS20Y/MS cells did not have an effect on the subcellular distribution of PKC.

PKC-mediated phosphorylation of endogenous protein substrates in vitro was studied next. Cellular extracts were incubated with the PKC reaction mixture and [γ-32P]ATP as described in Methods and the phosphorylated proteins were separated by SDS-PAGE. In these experiments PKC-mediated phosphorylation was detected in both cell types; nonetheless, it was substantially higher in NS20Y/MS cells. In extracts of MV-infected cells intensely phosphorylated proteins ranging in Mr from 27K to 60K were observed (Fig. 2a, compare lane 1 with lane 2). To test whether phosphorylation was associated with viral proteins, MV proteins were immunoprecipitated from NS20Y/MS cell extracts using polyclonal rabbit anti-MV antibodies. No phosphorylated viral proteins were detected (data not shown).

To test whether the augmented activity of PKC is reflected by an increase in PKC synthesis, immunoblotting was performed with polyclonal anti-PKC or monoclonal anti-PKCα antibodies. The results, shown in Fig. 2(b) and (c) respectively, revealed a significant increase in the detected level of the 80K PKC proteins expressed in NS20Y/MS cells with both antibodies.

Downregulation of total protein phosphorylation activity in NS20Y/MS cells by anti-MV antibodies

In the following experiments the effect of anti-MV antibody treatment on total phosphorylating activity in cytosolic fractions of NS20Y/MS cells was investigated. Cells were grown in the presence of polyclonal anti-MV antibodies under conditions shown to downregulate MV protein synthesis (Schneider-Schaulies et al., 1992). A decrease in total phosphorylating activity was first
Fig. 3. Downregulation of total protein phosphorylation by anti-MV antibodies. Cells were grown in the presence of polyclonal anti-MV antibodies (solid line). Total phosphorylation activity was determined at different times during antibody treatment in the cytosolic fraction by using a standard kinase assay. Recovery of the level of total protein phosphorylation following growth in the absence of antibody is indicated by a broken line. Data are representative of three independent assays.

Fig. 4. Downregulation of PKC activity by anti-MV antibodies. Cells were grown in the presence of polyclonal anti-MV antibodies. PKC activity was determined, at various times during antibody treatment, in the cytosolic fraction as described in Methods. PKC activity in NS20Y cells was taken to be 100%. (A) untreated NS20Y/MS cells; (B) 2 days and (C) 6 days of growth in the presence of antibodies; (D) 6 days with antibodies followed by 14 days growth in the absence of antibodies. The results are the mean of four independent experiments. In all cases these determinations did not differ by more than 10%. (□) NS20Y cells, (■), NS20Y/MS cells.

detected after 2 days of treatment with anti-MV antibodies and reached the baseline levels of uninfected cells after 4 to 6 days (Fig. 3). This effect was reversible since the original level of phosphorylating activity of NS20Y/MS cells was gradually restored when the cultures were grown in fresh medium without antibodies. Treatment of the cells with either anti-H or anti-N protein monoclonal antibodies or polyclonal antiserum non-specific for MV had no such effect.

Downregulation of PKC activity in NS20Y/MS cells by anti-MV antibodies

Next we investigated whether the enhanced PKC activity in NS20Y/MS cells could also be modulated by treatment with anti-MV antibodies. As was mentioned above, PKC activity was higher in NS20Y/MS than in NS20Y cells. Reduction in PKC activity was observed on the second day of antibody treatment (Fig. 4, compare B with A) reaching the level of PKC activity in NS20Y cells by day 6 (Fig. 4, C). Removal of the antibodies and growth of the cells for an additional 14 days resulted in the restoration of PKC activity almost to the original level of NS20Y/MS cells (Fig. 4, D). Treatment of the cells with monoclonal anti-H or anti-N protein antibodies had no such effect (data not shown).

Inhibition of MV release by H-7

To study the involvement of PKC in the life cycle of MV, the release of infectious virus from NS20Y/MS cells was measured after treatment with H-7. NS20Y/MS cells were cultured in the presence or absence of 100 μM-H-7 for 24 h. As can be seen from Table 2, the yield of infectious virus was strongly inhibited, decreasing from 1.5 x 10⁶ to 7 x 10⁴ p.f.u. in the presence of H-7. The compound had no effect on the level of MV proteins as measured by Western blot analysis of cell extracts (data not shown). These results may indicate that there is an intimate relationship between PKC-mediated phosphorylation and the assembly and budding of infectious MV particles.

Discussion

Clone NS20Y/MS of the murine neuroblastoma C1300 was infected with the wild-type Edmonston strain of MV and, after transition to a carrier culture, became

Table 2. Effect of H-7 on MV release by NS20Y/MS cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Treatment</th>
<th>Virus release*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS20Y</td>
<td>None</td>
<td>1.5 x 10⁶</td>
</tr>
<tr>
<td>NS20Y/MS</td>
<td>H-7</td>
<td>7 x 10⁴</td>
</tr>
<tr>
<td>NS20Y/MS</td>
<td>H-7</td>
<td>7 x 10⁴</td>
</tr>
</tbody>
</table>

* Cells were incubated with 100 μM-H-7 for 24 h. Virus release, expressed in p.f.u./ml, was determined by plaque formation on Vero cells, as described in Methods.
persistenty infected (Rager-Zisman et al., 1984). The cultures maintained a morphology indistinguishable from that of the parental uninfected NS20Y cells; nonetheless, immunofluorescence studies revealed accumulation of viral proteins in the majority of cells. We have previously investigated the effect of persistent MV infection of NS20Y cells and have shown that NS20Y/MS cells are less tumorigenic and express enhanced amounts of major histocompatibility complex class I glycoproteins (Gopas et al., 1992). In addition increased expression of the c-fos proto-oncogene and PKC genes was also detected (Wolfson et al., 1989, 1991).

In the present report we demonstrate that an intimate relationship exists between the synthesis of MV proteins and an increase in the phosphorylating activity as detected in the persistently infected cells. The endogenous phosphorylating activity in NS20Y/MS cells was 50% higher than that in the uninfected cells. The increase in total protein phosphorylation suggests that several kinases may be activated by MV. In this context we have recently shown that the activity of a dsRNA-dependent kinase was significantly elevated in NS20Y/MS cells (Gopas et al., 1992). We have shown previously that the increase in PKC activity paralleled the elevation of PKC gene expression (Wolfson et al., 1989, 1991). Here we confirm this observation at the protein level, demonstrating an increase in the 80K protein detectable by using anti-total PKC or anti-PKCα antibodies. It appears that MV requires a PKC-mediated phosphorylation step as shown for other viruses including human immunodeficiency virus (Chowdhury et al., 1990; Kinter et al., 1990) and bovine leukaemia virus (Jensen et al., 1992).

In the present study we show that although the extent of phosphorylation of endogenous proteins was consistently higher in NS20Y/MS cells, differences between the protein substrates could not be detected, indicating that these were primarily cellular proteins. In contrast, when PKC-mediated phosphorylation was examined, additional species of phosphorylated proteins could be observed in NS20Y/MS cells. The Ms of these proteins were between 30K and 80K, similar to those of MV proteins. The results of immunoprecipitation with anti-MV antibodies were negative suggesting that the phosphorylated proteins are of cellular origin and that their phosphorylation is induced by the virus. Although it is well established that MV proteins P, N and V are phosphorylated, from this study it appears that PKC is not involved in this process. Another serine kinase, such as casein kinase II, shown by Barik & Banerjee (1992) to phosphorylate the P protein of vesicular stomatitis virus, is a probable candidate. The strong correlation found between the high MV protein content and both Ca2+/lipid-dependent or -independent phosphorylating activity may indicate that MV infection can directly or indirectly mediate the activity of these kinases. The mechanism underlying this effect is as yet unknown although it has been described for a variety of DNA and RNA viruses, including polyoma virus, hepatitis B virus, adenovirus, herpes simplex virus, and human T cell leukaemia virus types I and II (De Wind et al., 1992; Giordano et al., 1991; Nevins, 1989, 1991; Shiroki et al., 1992; Suzuki et al., 1992; Tan et al., 1989; Yoakim et al., 1992; Maguire et al., 1991; Wu et al., 1990; Can & Chen, 1990).

We have shown recently that binding of polyclonal antibodies against MV or a mixture of monoclonal anti-H protein antibodies to NS20Y/MS cells resulted in downregulation of MV mRNA and protein synthesis (Schneider-Schaulies et al., 1992). When the effect of this treatment on total phosphorylation or PKC activity was examined, downregulation occurred only when polyclonal antibodies were used. Treatment of the infected cells with antibodies against the intracellular N protein also had no such effect and in this case it appears that binding to a membrane entity is required. Furthermore it also appears that, in order to downregulate total phosphorylation or PKC activity, binding to more than one viral epitope is required since treatment with a single anti-H monoclonal antibody that binds and neutralizes MV had no effect. In this context, it is worth noting that monoclonal antibodies that bind to the same antigen may recognize epitopes with different structures since their binding sites are distinct in shape (Simpson et al., 1993). Therefore, it seems possible that binding to more than one epitope is essential for inducing the conformational changes that will trigger the intracellular signalling.

The nature of the transduction pathway involved in the delivery of the signal triggered by the binding of anti-MV antibody is still unknown. Weinemann-Dorsch & Koschel (1989) reported that treatment of rat glioma cells, persistently infected with the SSPE strain of MV, with anti-MV antibodies stimulated phosphatidylinositol breakdown and inositol phosphate formation (Weinemann-Dorsch & Koschel, 1989; Tas & Koschel, 1991). Alterations in PIP2 metabolism do not necessarily result in the activation of PKC, and because in these experiments PKC activity was not measured it is difficult to compare the results of both systems.

Modulation of viral protein synthesis and cellular phosphorylation by anti-MV antibodies in NS20Y/MS demonstrates that there is a dynamic equilibrium between the persistent virus state and cellular metabolism. Viruses often use cellular phosphoprotein kinases to phosphorylate their own proteins in order to activate their functions. Examples of this include potentiation of the v-fms transforming potential of feline sarcoma virus
by phosphorylation on threonine-939, phosphorylation of the v-erb A on serine 16/17 which is essential for its oncogenic potential, and reactivation of herpes simplex virus type I from latency by PKC activators (Glineur et al., 1990; Leib et al., 1991; Tamura et al., 1991). Although MV neither transforms the cells nor causes immortalization, phosphorylation may be a necessary step in its replication. It was indeed reported that TPA, which strongly activates PKC, was a very strong inducer of productive MV infection in peripheral blood monocytes (Vaniopäa et al., 1991). These results are in agreement with our observation that treatment with H-7 decreased the release of infectious virions. Treatment of NS20Y/MS with H-7 for 24 h led to a profound inhibition of the release of infectious MV. This suggests that phosphorylation mediated by PKC and/or other kinases such as protein kinase A, is an essential step in assembly and maturation of the virions.

In conclusion, the results presented here suggest that the augmented PKC activity is a necessary step in the maintenance of the persistent infection as well as in viral maturation. It is proposed that viral antigens expressed on the host cell membrane behave as receptors transducing signals to maintain viral replication without affecting cell viability.

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