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Effect of persistent measles virus infection on protein kinase C activity and c-fos protooncogene expression in neuroblastoma cells

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The effect of persistent measles virus infection on c-fos protooncogene and protein kinase C (PKC-I) gene expression in a murine neuroblastoma cell line was studied. Overexpression of c-fos protooncogene by infected NS20Y/MS cells was detected when compared with uninfected NS20Y cells. The level of PKC-I-specific mRNA was increased in infected NS20Y/MS cells. In addition, the level of total PKC activity in these cells was also enhanced. We conclude that persistent measles virus infection can alter both protooncogene expression and signal transduction in cells of neuronal origin.

Protein kinase C; Protooncogene, c-fos; (Murine neuroblastoma C1300)

1. INTRODUCTION

There are many infections in which viruses are not eliminated from the body but persist in the host for months, years or a lifetime. Persistent infections cannot by definition be acutely lethal; they tend to cause mild damage or disease in the host or produce disease only when reactivation of latent genomes occurs. The central nervous system (CNS) appears to be uniquely predisposed for the development of such infections. The structural organization, lack of a lymphatic system, intense cellular specialization and high metabolic requirements, may in part explain why viruses tend to persist in the CNS [1].

Among the infectious agents displaying a propensity for establishing persistent infections of the CNS is measles virus. This virus has been isolated from patients with subacute sclerosing panencephalitis (SSPE) [2] and may be involved in the pathogenesis of other neurological disorders of unknown etiology, such as multiple sclerosis [3].

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Measles virus is capable of infecting various kinds of cells in culture, causing an acute lytic effect, while resulting in nonlytic, persistent infections of murine neuroblastoma cells or spinal cord cultures [4,5]. The available experimental and clinical data suggest that specific interactions may exist between the invading measles virus and the CNS. The mechanism of measles virus persistence is unknown, but most probably involves alterations in both cell metabolism and gene expression. Among the genes whose expression may be potentially affected by measles virus infection are some protooncogenes playing an important role in neuronal cell differentiation and functions [6]. For instance, stimulation of neuronal cells by neurotransmitters, nerve growth factor, phorbol esters or synaptic activity in most cases results in rapid and transient expression of the c-fos protooncogene. Thus, the c-fos protein product is among the early inducible regulatory proteins synthesized in response to cell differentiation agents [7]. Studies on the molecular mechanism leading to the expression of c-fos suggest that the inductive signals are delivered to the DNA transcription machinery by different transduction pathways [8,9], such as phosphatidylinositol (PI)/protein

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kinase C (PKC) which is the major transducing pathway in neuronal cells [10]. Activation of PKC by serum growth factors or phorbol esters results in rapid expression of the c-fos gene [11,12]. The present experiments were undertaken in order to investigate whether persistent measles virus infection of cell lines derived from the CNS will influence protooncogene expression and signal transduction. The results indicate that both c-fos expression and the PKC signalling pathway of murine neuroblastoma cells are altered by persistent measles virus infection at several levels.

2. MATERIALS AND METHODS

2.1. *Cells*

The mouse neuroblastoma C1300, clone NS20Y and clone NS20Y/MS persistently infected with the Edmonston strain of measles virus were used [13]. Cells were grown as loosely adherent monolayers in RPMI medium supplemented with 10% fetal calf serum (FCS), glutamine and antibiotics.

2.2. *Northern blot analysis*

Poly(A)$^+$-enriched fractions from both cell lines were prepared according to [14]. Poly(A)$^+$ mRNAs were electrophoresed and blotted according to the Northern protocol [15] and hybridized to dCM^{32}P-labeled nick-translated *Eco*RI plasmid fragments. The c-fos DNA probe was provided by Dr I. Verma [16] and the PKC-I probe by Dr I. Weinstein (Columbia University, NY) [17]. The position of the bands was determined by autoradiography.

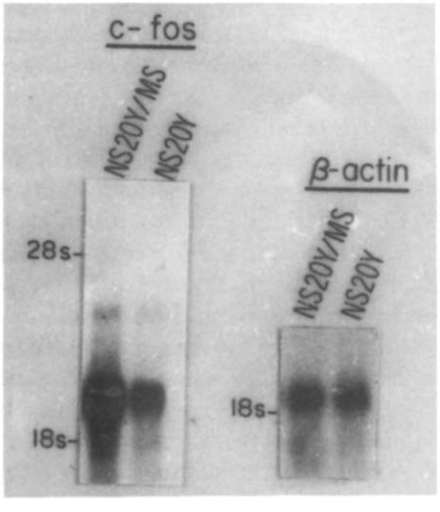

Fig.1. Expression of c-fos mRNA transcripts in infected NS20Y/MS and uninfected NS20Y neuroblastoma cells, determined by Northern hybridization analysis (A). The same mRNA preparations were probed with B-actin cDNA (B). Size markers are 18 S and 28 S ribosomal RNA.

2.3. *Preparation of cell fractions and assay of protein kinase C*

Cell monolayers were lysed in extraction buffer containing 20 mM Tris-HCl (pH 7.5), 0.5 mM EGTA, 1 mM PMSF, 10 μg/ml each of leupeptin and aprotinin and 50 mM 2-mercaptoethanol. Extraction of PKC from the membrane pellet or whole cell homogenates was performed in the same buffer with 1% Triton X-100. After sedimentation at 30000 × *g* for 30 min, cytosolic fractions or whole cell extracts were purified by DEAE chromatography and used for measurements of PKC activity [18,19].

3. RESULTS

Expression of the c-fos protooncogene in both cell clones was studied. The results (fig.1) clearly show that the virus-infected cells (NS20Y/MS) expressed considerably higher levels of c-fos mRNA than did uninfected NS20Y cells.

When total enzymatic PKC activity was measured in the extracts of NS20Y/MS or uninfected NS20Y cells, it appeared that per-

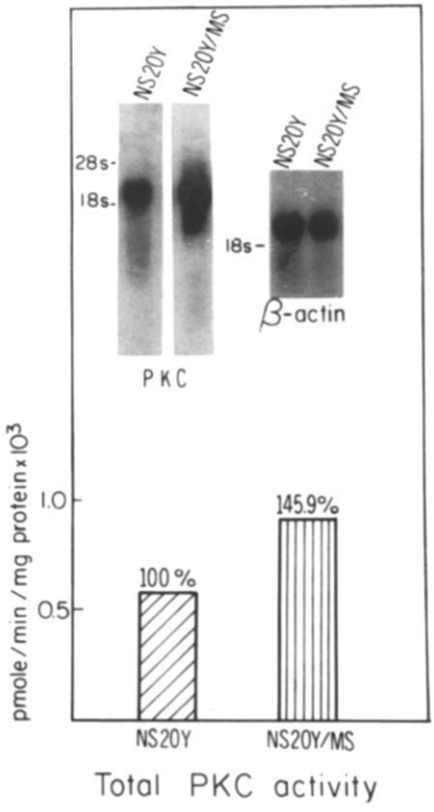

Fig.2. Expression of PKC-1 specific mRNA (A) and total PKC activity (B) as detected by Northern analysis in infected NS20Y/MS and uninfected NS20Y/MS cell clones. Histograms represent mean of 6 experiments ± SE.

Table 1

PKC activity in persistently measles-infected neuroblastoma cells (pmol/min per mg protein)

Cell fraction	NS20Y	NS20Y/MS	Increase (%)
Cytosolic	298 ± 76[a] (55%)[b]	469 ± 91 (60%)	57.3
Particulate	240 ± 125 (45%)	316 ± 155 (40%)	31.6

[a] SE

[b] % of total PKC activity

Neuroblastoma cells (5×10^7) were fractionated as described in section 2. Cell fractions were chromatographed on DE52 cellulose columns (0.9×2 cm) and the PKC activity determined in 50-μl aliquots (0.5–0.8 mg/ml protein)

sistently infected cells showed a 50% increase in PKC activity vs uninfected cells (fig.2B). Determination of the PKC activity in cytosolic and particulate fractions revealed that the increase was almost equally distributed between both fractions, being slightly higher in the cytosolic fraction (table 1). The subcellular distribution of PKC activity expressed as the cytosolic/particulate ratio was practically unchanged.

We next analyzed the level of PKC mRNA in both cell lines. Northern blotting experiments using poly(A)$^+$-enriched mRNA and specific PKC-I probe demonstrated that the infected clone, NS20Y/MS, expressed an elevated level of PKC-I mRNA transcripts which correlated well with the greater PKC activity in these cells (fig.2A).

4. DISCUSSION

Viral diseases in man are frequently complicated by viral infections of the CNS. The CNS represents a unique site for the establishment of such infections because of its anatomical and functional characteristics [1]. Many viral diseases are characterized as neurological disorders (Kuru, Creutzfeld-Jacob disease, SSPE, progressive rubella, panencephalitis, etc.) [20] and it has recently been shown that neurons are the first target for HIV infection and that neurological symptoms appear before the immunodeficiency syndrome [21].

Here, we showed that persistent measles infection resulted in the alteration of two important molecular events in cells of neuronal origin, c-fos protooncogene expression and PKC-associated signal transduction. Our results suggest that measles virus is capable of up-regulating c-fos and PKC gene transcription. Measles virus was not shown to integrate into the host cell genome. Instead, persistently infected cells contain large amounts of the viral nucleoprotein matrices and other viral proteins [4]. The effect of accumulated measles viral proteins on cell metabolism and function remains unknown. One may speculate that it might result in the trans activation of PKC or c-fos genes [22] by some viral proteins acting on regulatory elements of these genes. Alternatively, the accumulation of viral proteins in the cytoplasm and nucleus may alter the turnover of PKC and c-fos mRNAs. In any case, the overproduction of PKC may be compensatory to the infected cell by increasing its responsiveness to regulatory signals, mostly acting through the PI/PKC pathway. The alterations in signal transduction pathways caused by measles virus may be a general phenomenon, since alteration in the cAMP-dependent signalling pathway has also been reported for measles-virus infected rat glioma cells [26]. The increase in c-fos protooncogene expression may or may not correlate with the increase in PKC activity, since at least one more pathway involving adenylate cyclase was reported [9]. The c-fos protein product is a nuclear DNA-binding phosphoprotein which binds to the regulatory sequences of the responsive genes [24,25]. Binding of the known transcription factors to the same regions on DNA suggests that the c-fos gene product acts as a transcriptional regulator. Under normal physiological conditions, it is rapidly turned off, triggering the transcription of later genes. c-fos has been directly implicated in normal metabolic functions such as cell growth, differentiation and function. However, the normal c-fos gene product transforms fibroblasts in vitro when the regulation of the fos gene is broken [7]. It has also been shown that a wide variety of human tumors contain high amounts of the c-fos mRNA. In addition, an increase of c-fos mRNA in acutely infected quiescent BALB/C 3T3 mouse cells by polyoma virus was reported [26].

Our results demonstrate that in persistently infected cells, the c-fos gene is expressed in large quantities compared to uninfected cells. Considering the enormous variety of human diseases of unknown etiologies, it is tempting to speculate that

some are caused by deregulation in gene expression and signal transduction as a result of a persistent virus infection. If such deregulated activity of the c-fos gene or other pivotal genes is observed in additional persistently virus infected cells of CNS origin, both in vitro and in vivo, it may help to delineate some of the currently unknown mechanisms by which such infections may impair the normal physiological functions of a neural cell.

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