The effects of measles virus persistent infection on AP-1 transcription factor binding in neuroblastoma cells

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Abstract Measles virus (MV) persistence in brain cells has broad effects on different cellular functions. We have previously shown that NS20Y clone, originally derived from C1300 neuroblastoma cells, persistently infected with MV (NS20Y/MS), displays constitutively elevated levels of c-*fos* and PKC mRNAs, implying MV-mediated effects on transcriptional regulation. Nonetheless, the mode by which virus affects the transcriptional machinery still remains obscure. In order to define this phenomenon, we studied the binding properties of major transcription factors (AP-1 and NFκB) in NS20Y/MS cells. Using electrophoretic mobility shift approach (EMSA) with the appropriate oligonucleotide probes, we have found that the persistent MV infection does not affect NFκB binding, while the AP-1 binding was significantly decreased. Similar inhibition was not observed in NS20Y cells acutely infected with MV. Anti-measles antibody-mediated restriction of viral gene expression restored AP-1 binding, thus suggesting that measles virus proteins may affect the components of the host transcriptional machinery.

Key words: Measles virus; Persistent infection; AP-1; Fos; Jun; Neuroblastoma cell

1. Introduction

Measles virus (MV) is a common human pathogen responsible for an acute epidemic disease with a worldwide prevalence. It is also associated with subacute sclerosing panencephalitis (SSPE) and measles inclusion bodies encephalitis (MIBE) that occur months to years after onset of acute measles on the basis of MV persistence in brain cells [1]. Although the molecular basis of persistent infection was characterized by alterations of viral gene expression on the transcriptional and translational levels and morphological pictures of the both SSPE and MIBE were described, little is known about the molecular basis of MV-mediated cytopathic effects in the brain [2–4].

We have previously demonstrated that the persistent infection of murine neuroblastoma cells with MV resulted in a spectrum of changes in the expression of cellular genes, i.e., an enhanced transcription of c-*fos* and PKC genes, a diminished level of Ha-ras mRNA [5] and elevated level of MHC class I glycoproteins on plasma membrane [6]. These observations imply MV-mediated effects on gene expression in host cells.

Regulation of gene expression in nucleus occurs via modulation of transcription factors activity. The ubiquitous transcription factor, activating protein 1 (AP-1), regulates the transcription of a variety of genes. AP-1 is constituted by the products of Jun family proteins, including c-Jun, Jun-B and Jun-D, and the products of Fos family (c-Fos, Fos-B, Fra-1 and Fra-2) [7]. In response to the appropriate signal, such as phorbol esters, growth factors and other mitogenic agents, activated Fos/Jun or Jun/Jun dimers bind to the palindromic sequence TGAC/GTCA present in the promoters of a number of genes induced by phorbol esters and, thereby, known as TPA responsive element (TRE) [8,9]. Functional specificity of different Fos and Jun families is most likely determined by their differential distribution and inducibility. These proteins act as cell-specific nuclear third messengers converting cytoplasmatic signals into long-term adaptive changes in cell phenotype by regulating the expression of specific target genes [7].

c-Fos is induced in many different cell types by a wide variety of signals and is involved in cell proliferation, differentiation, neuronal excitation and other basic processes [10–12]. Different receptor–second messenger systems can modulate the composition of Fos/Jun dimers thereby regulating the expression of distinct subsets of genes. Most neuronal tissues express low basal levels of AP-1 transcription factors, which dramatically increase after a strong stimuli [12]. The induction of Fos and Jun proteins was well studied in striatum and hyppocampus, AP-1 was shown to be involved in regulation of circadian pacemaking system, in modulation of the behavioral rhythms, in biochemical processes underlying learning and memory [13–15]. Given the marked elevation in c-*fos* mRNA in MV persistently infected cells, we wished to determine whether these changes will affect DNA-binding capacity of AP-1 transcription factor complex. Here we present the results describing the effect of the persistent MV infection on the level of Fos and Jun proteins and on the DNA-binding activity of AP-1 transcription regulatory complex in persistently infected cells of neuronal origin.

2. Materials and methods

2.1. Cells lines

The C1300 neuroblastoma, clone NS20Y, of the A/J mouse strain was used. The establishment of MV persistently infected NS20Y cell clone, termed NS20Y/MS, was described in detail elsewhere [16]. Uninfected or infected cells were routinely grown in Dulbecco's modification of Eagle's tissue culture medium (DMEM; Beit Haemek, Israel) supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% glutamine and 1% antibiotics. For acute infection, NS20Y cells were infected with 5×10^4 pfu of the Edmonston strain of MV and harvested 72 h later as previously described [17].

2.2. Antibodies

The following commercial monoclonal antibodies (mAb) were used: c-Fos, FosB, Fra-1, Fra-2, c-Jun (Oncogene Sci., Uniodale, NY, USA), JunB, JunD (Santa Cruz Biotechnology, Santa Cruz, CA, USA). mAbs against measles virus nucleocapsid protein (MV N)

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were described by Birrer et al. [18]. Anti-MV H mAb (hybridoma clones: L77, K83 and NC32) and anti-MV N were raised and kindly provided by V. ter Meulen [19,20]. Anti-MV N monoclonal antibody was a gift from Dr. V. ter Meulen.

2.3. Anti-MV antibody treatment of cells

For antibody (Ab) treatment, the medium of semiconfluent cultures was replaced with fresh medium containing a mixture of monoclonal neutralizing anti-H mAbs (L77, NC32, and K83), sufficient to neutralize 100 pfu of MV in a conventional Vero cell neutralization assay [21]. The cells were split once every 3 days and fresh mAb-containing medium was added. Untreated and Ab-treated cells were harvested at different time intervals after initiation of Ab treatment, and cell lysates were prepared.

2.4. Preparation of total cell lysates

Cells were washed by phosphate-buffered saline (PBS) and lysed in boiling lysis buffer (10 mM Tris-HCl (pH 8), 1% SDS). Following additional boiling for 5 min, lysates were passed several times through a needle and clarified by centrifugation at $12000 \times g$ for 5 min.

2.5. Western blot analysis

Cell lysates were mixed with Laemmli sample buffer and boiled for 5 min. Protein samples containing equal amounts of protein (50 μg) were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to Western blotting analysis in the following manner. The resolved proteins were transferred to nitrocellulose membrane (Schleicher and Schuell, Keene, NH, USA), non-specific binding sites were blocked with 5% low-fat dry milk in PBS containing 0.05% Tween-20 for 1 h at room temperature, followed by incubation with primary antibody. The strips were washed in PBS–0.05% Tween-20 and incubated with peroxidase-conjugated secondary antibody (Amersham Life Sciences, Amersham, UK) for an additional 1 h. After washing in PBS–0.05% Tween-20, immunoreactive proteins were visualized using enhanced chemiluminescence kit (Amersham Life Sciences).

2.6. Oligonucleotides and electrophoretic mobility shift assay (EMSA)

The following consensus sequence oligonucleotides were used in this assay: monomeric TRE:5′-CGCTTGATGACTCAGCCGGAA-3′ (Santa Cruz Biotechnology), TRE tetramer: 5′-TGACTCA TGACTCA TGACTCA TGACTCA-3′ (kindly provided by Dr. P. Kourilski), TRE-like consensus sequence derived from murine c-*jun* promoter: 5′-GATCCCTCGGGTGACATCACTAG-3′ [22] and NFκB consensus sequence derived from human IL-2 promoter: 5′-GATCCGAAAGAGGGATTTCACCTG-3′ [23]. Preparation of nuclear lysates and electrophoretic mobility shift assay were performed as described elsewhere [24].

3. Results

The expression of the Fos proteins (c-Fos, Fos-B, Fra-1 and Fra-2) and the Jun family proteins (c-Jun, Jun-B and Jun-D) was analyzed by Western blotting with the appropriate monoclonal antibodies. A slight increase in the expression of c-Fos was detected in the total cell lysates of persistently infected NS20Y/MS cells (Fig. 1). No differences in the expression of either Fos-B or Fra-1 proteins were observed in total cell lysates of NS20Y/MS or NS20Y cells. Fra-2 protein was undetectable in both cell lines. Jun-B protein expressed at relatively high level in NS20Y cells was significantly down-regulated in NS20/MS. No differences in the levels of Jun-D and c-Jun were observed between NS20Y/MS and NS20Y cells (Fig. 1).

To investigate whether the alterations in the expression of c-Fos and Jun-B proteins in persistently infected NS20Y/MS cells will affect the DNA-binding capacity of the AP-1 (Fos/Jun) complex, electrophoretic mobility shift assay was performed using three different TRE consensus oligonucleotides. The results clearly show that the binding of AP-1 to the monomeric TRE oligonucleotide (TRE monomer) was significantly decreased in the persistently infected NS20/MS cells (Fig. 2A). This decrease became more profound when an oligonucleotide representing the tetramer of TRE was used. No such decrease was observed when the oligonucleotide representing TRE-like element derived from the c-*jun* promoter was used. This sequence slightly differs in sequence from TRE and binds heterodimers of c-Jun and cAMP responsive element binding (CREB) family member, i.e., ATF-2 [25]. Competition with the excess of the unlabeled monomeric TRE oligonucleotide confirmed specificity of the binding (Fig. 2B). DNA binding capacity of another transcription factor, NFκB, was not affected by MV persistence (Fig. 2C). We have previously shown that the treatment of NS20Y/MS cells with anti-HA-MV antibodies resulted in down-regulation of MV mRNA and MV protein synthesis. To establish a linkage between the persistent infection and inhibition of AP-1 binding, we treated NS20Y/MS cells with anti-HA-MV antibodies. As depicted on Fig. 3A, this treatment restored AP-1 binding with concomitant decrease in the levels of MV N protein. To investigate whether the effect of MV on AP-1 binding is characteristic to the persistent state, we tested the binding of nuclear proteins to the TRE tetramer in NS20Y cells acutely infected with MV. The synthesis of MV N protein was confirmed by Western blotting analysis with monoclonal anti-MV N antibodies. We observed an increase in AP-1 binding in acutely infected cells comparing to the uninfected control in contrast to the marked decrease of AP-1 binding in persistently infected NS20Y/MS cells (Fig. 3B).

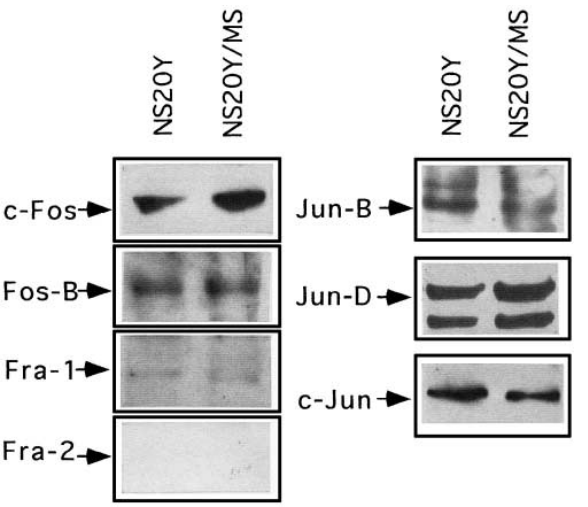

Fig. 1. The expression of AP-1-related proteins in neuroblastoma cells persistently infected by MV. Total cell lysates of NS20Y/MS and NS20Y cells were prepared as described in Section 2 and subjected to SDS-PAGE (50 μg/lane, each) followed by Western blotting analysis with the appropriate antibodies.

4. Discussion

In this study we examined the effect of MV persistence on AP-1 factor DNA-binding capacity. Establishment of viral persistency is accompanied by alterations in the level of a number of proteins, both cellular and viral [5,6,26,27]. In regard to the transcriptional factors, it means that any change in the level of these proteins may influence the composition of the active transcription complexes and, consequently, affect the affinity to the specific binding sites on DNA. Viruses can affect an activity of a variety of cellular transcription

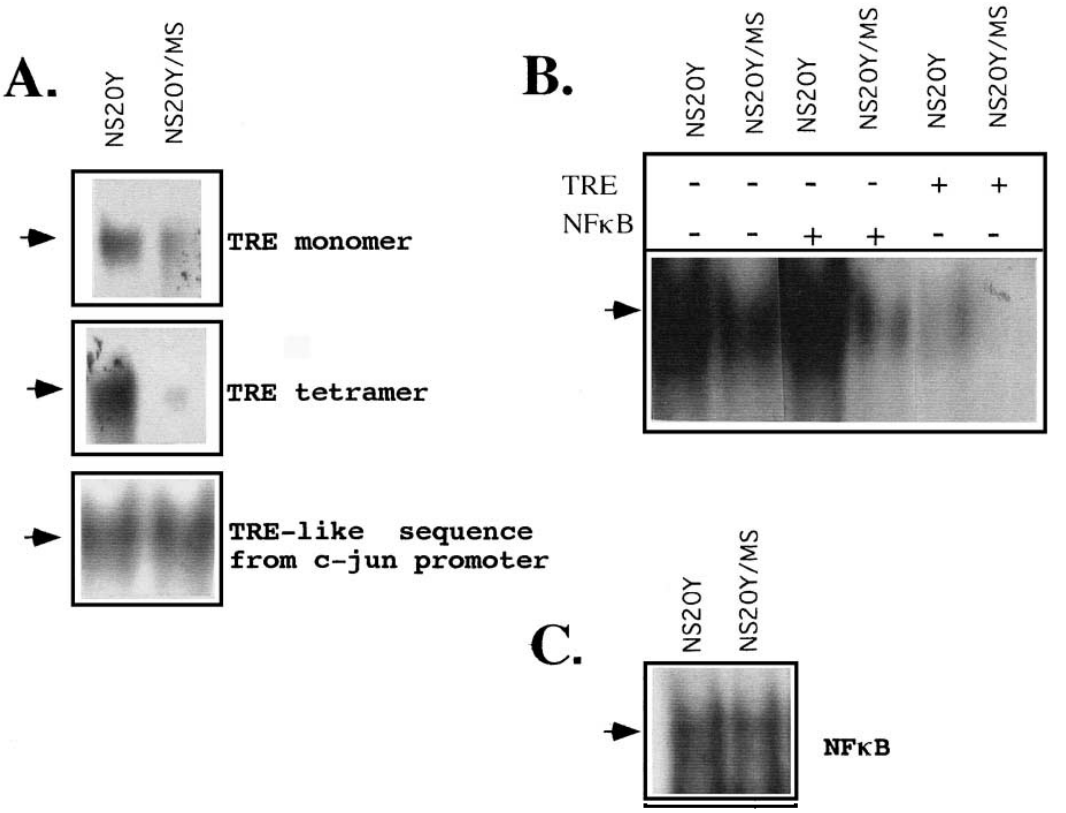

Fig. 2. AP-1 binding is inhibited in NS20Y/MS cells. Nuclear lysates of NS20Y/MS and NS20Y cells were prepared as described [24] and analyzed by EMSA. A: Five μg of each extract was incubated with [^{32}P]γ-ATP end-labeled TRE probes. The reaction mixtures were resolved on 5% PAGE, the gel was dried and exposed to X-ray film. B: Competition with 50 molar excess of unlabeled specific TRE monomeric and unlabeled non-specific NFκB probes. C: Nuclear lysates (5 μg) were incubated with [^{32}P]γ-ATP end-labeled NFkB probe and subjected to EMSA.

factors and AP-1 complex is one of the major targets [28–30]. AP-1 DNA-binding activity is enhanced by Tax protein of HTLV-1 [31]. The hepatitis virus B transactivator protein Hbx stimulates a variety of cellular promoters through the increase in binding activity of AP-1 (Fos/Jun), AP-2 and NFκB transcription factors [32]. This strategy of an altered DNA-binding specificity by viruses may result in the modification of a repertoire of cellular genes expressed during viral infection and affect functional activity of the infected cells.

We have previously demonstrated that the level of c-*fos* mRNA is elevated in the persistently infected cells [5]. Our present results indicate that the level of c-Fos and Jun-B pro-

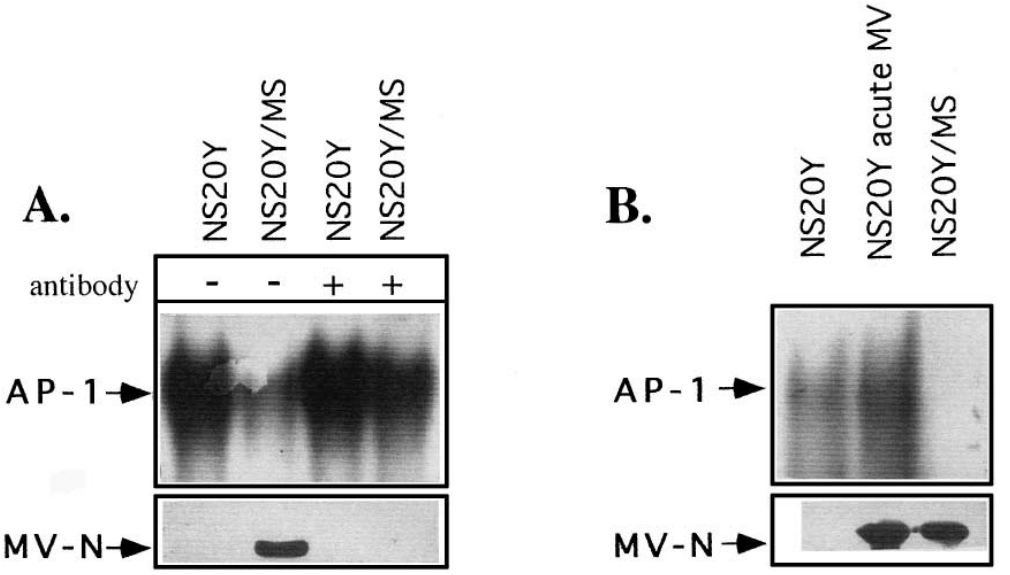

Fig. 3. Decreased AP-1 binding is characteristic to MV persisitence in neuroblastoma cells. A: NS20Y/MS and NS20Y cells were cultured for 21 days in the presence of a mixture of three anti-MV H antibodies as described in Section 2. Nuclear lysates prepared from Ab-treated and untreated cells were subjected to EMSA (5 μg/lane, each) with [^{32}P]γ-ATP end-labeled TRE tetrameric probe. Total cell lysates prepared from Ab-treated and untreated cells were subjected to Western blotting analysis (50 μg/lane, each) with monoclonal anti- MV N antibody. B: NS20Y cells were acutely infected with MV as described in Section 2 and AP-1 binding was analyzed by EMSA with TRE tetramer as above.

teins were altered in the neuroblastoma cells persistently infected with MV. Despite extensive data on the expression of c-*fos* in a variety of cells, its role is not fully understood. Rapid induction of c-*fos* in the cells of neuronal origin in response to nerve growth factor, sensory stimulation and chemical agents indicates that it plays an important role in the responses of neuronal cells [33,34]. A number of nuclear proteins may bind c-Fos resulting in AP-1 complexes with different specificity. Regulatory systems which determine the exact composition of AP-1 in each particular situation are highly complex.

AP-1 (Fos/Jun) binding to the TRE consensus sequence was markedly decreased in neuroblastoma cells persistently infected neuroblastoma cells. This decrease was specific to the TRE consensus site since binding to the similar, but not identical, sequence originated from a c-*jun* promoter, which binds c-Jun/ATF-2 dimers, was not changed. Down-regulation of the MV mRNA and MV protein levels by neutralizing anti-MV-HA antibodies in persistently infected cells restored TRE binding (Fig. 3A). The decrease in TRE binding may be a consequence of an accumulation of MV proteins in the infected cells that affects the formation of different AP-1 dimers. Alternatively, it may be a manifestation of an adoptive response of the cell to the persistent viral infection. To answer this question, we tested AP-1 binding in neuroblastoma cells acutely infected with MV. As can been seen from Fig. 3B, TRE binding was increased in acutely infected cells comparing to uninfected controls, in contrast to persistently infected cells which showed diminished AP-1 binding. However, both acutely and persistently infected cells expressed MV N protein at the comparative levels. Thus, the decrease of binding of nuclear proteins to the TRE consensus oligonucleotide appears to be the characteristic feature of the adaptive response of neuroblastoma cells to MV persistence. Alterations in the DNA-binding capacity, observed in persistently infected cells, were specific to the TRE element since no changes in NFκB were found.

The molecular pathogenesis of the diseases associated with MV persistence in brain cells is poorly understood. No infectious virus is formed in the persistent state and large amounts of nucleocapsid particles are accumulated in the cytoplasm [16]. We suggested that dwelling of a foreign substance such as virus or viral proteins in a cell cannot be inert. Rather, it starts as a cascade of pathological metabolic changes which are clinically 'silent' but develop into a serious disorder over time and eventually lead to the clinical manifestation characteristic to SSPE, MIBE or other cerebral dysfunction. Noteworthy, some clinical and pathological features of Alzheimer disease show a resemblance to viral infections [35–37].

A tissue culture model of neuroblastoma cell persistently infected with measles virus developed in our laboratory confirms the above prediction that the viral persistence affects intracellular communication events including gene expression [5,6,21]. Our results demonstrate that MV persistence is characterized by changes in the level of AP-1 constituents, Fos and Jun, and by alterations in binding of nuclear complexes to the TRE element. These alterations may lead to the changes in the regulation of a variety of TRE-containing genes and may be characteristic feature of the adaptive response of the cell to the persistent viral infection.

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