Protein Kinase Regulates Tumor Necrosis Factor mRNA Stability in Virus-stimulated Astrocytes

By Andrew P. Lieberman, Paula M. Pitha,* and Moon L. Shin

From the Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland 21201; and the *Oncology Center and Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Summary

Infection of astrocytes with Newcastle disease virus stimulated the production of 1,2-diacylglycerol, and resulted in the kinase-dependent expression of mRNAs encoding tumor necrosis factor (TNF), interferon α and β, and interleukin 6. The half-life of TNF mRNA was significantly decreased in the presence of protein kinase inhibitors H-7 and staurosporine, but not in the presence of HA1004. In contrast to the decay of TNF mRNA, the half-lives of other cytokine mRNAs were only minimally affected by the kinase inhibitors. These data indicated that the stability of TNF mRNA was regulated through a novel, kinase-dependent pathway.

Materials and Methods

Cell Culture and Stimulation. Primary cultures of rat astrocytes were established as described (5). Approximately 95% of the cells expressed the astrocyte marker glial fibrillary acidic protein and <2% expressed MAC-1. For induction, cells were stimulated with NDV, New Jersey LaSota strain, at a multiplicity of 30.

Measurement of 1,2-diacylglycerol. Astrocytes (8 x 10^5/60-mm dish) were incubated in serum-free DMEM/Ham's F12 for 30 min at 37°C and then stimulated with NDV. Reactions were terminated by the addition of ice-cold methanol. Measurement of cellular mass levels of 1,2-diacylglycerol and lipid phosphorus were performed as described (7). Data are reported as nanomoles of 1,2-diacylglycerol per 100 nmol of lipid phosphorus. There were ~55 nmol of lipid phosphorus per 8 x 10^6 cells.

Northern Blot Analysis. Total RNA was isolated and analyzed as described (6). Labeled RNA probes for mouse cytokines and IFN regulatory factor 1 (IRF-1) were generated using SP-6 and T7 promoter vectors. The TNF probe was constructed from a 1.1-kb fragment of TNF cDNA, the IFN-β probe from a 500-bp PstI fragment of IFN-β cDNA, the IFN-α, probe from a 776-bp EcoRI-BglII fragment of the IFN-α4 genomic clone, and the IRF-1 probe from a 1,024-bp Xbal-PvuII fragment of the IRF-1 cDNA. DNA probe for IL-6 was constructed using an oligolabeling reaction kit (Pharmacia Fine Chemicals, Piscataway, NJ) and a 650-bp EcoRl-BglII fragment of IL-6 cDNA.

Nuclear Run-On Assay. Nuclei (3 x 10^7) were isolated and nascent transcripts elongated in vitro as described (8). Labeled RNA was hybridized to denatured plasmids containing inserts for TNF, IRF-1, and β-actin that had been immobilized on nitrocellulose filters. pSV2-neo served as a control for nonspecific hybridization.
Results and Discussion

We began to explore the mechanism by which NDV induces cytokine mRNA accumulation by examining the role of signal messengers generated after cells were exposed to virus. NDV stimulated a transient production of 1,2-diacylglycerol in astrocytes that peaked within 1 min (Table 1). To determine whether activated protein kinases, particularly protein kinase C (PKC), participated in cytokine mRNA accumulation, cells were infected with NDV in the presence or absence of kinase inhibitors (Fig. 1). Infection of astrocytes with NDV in the presence of H-7 or staurosporine, two compounds that inhibit PKC (9, 10), prevented the accumulation of TNF, IFN-α, IFN-β, and IL-6 mRNAs in a dose-dependent fashion. In contrast, HA1004, a compound structurally related to H-7 but a relatively poor PKC inhibitor (9), failed to block cytokine mRNA accumulation. These results indicated that NDV-induced accumulation of cytokine mRNAs required PKC activity. Interestingly, accumulation of mRNA encoding IRF-1 was not affected by the kinase inhibitors. IRF-1 is a virus-inducible DNA binding protein that plays a positive role in the expression of IFN-β and possibly other cytokines (11). This finding confirmed that treatment of astrocytes with H-7 and staurosporine did not cause a nonspecific block of mRNA induction, and demonstrated that overexpression of IRF-1 mRNA can be dissociated from virus-induced expression of cytokine mRNAs.

To determine whether the kinase inhibitors affected NDV-stimulated transcription, run-on assays were performed with nuclei isolated from unstimulated astrocytes, and from cells stimulated with NDV or NDV in the presence of H-7 (Fig. 2). Virus infection increased TNF and IRF-1 transcription and concomitantly decreased actin transcription. A nontoxic dose of H-7 caused a partial inhibition of NDV-induced transcription of TNF and IRF-1. Although the transcription of these two genes was inhibited to a similar degree, H-7 had strikingly different effects on TNF and IRF-1 mRNA accumulation (Fig. 1). These findings suggested to us that a pathway involving protein kinases may regulate the stability of TNF mRNA in NDV-stimulated cells.

The role of kinases in regulating cytokine mRNA half-life was examined by infecting astrocytes with NDV for 6–8 h, and then adding α-amanitin at a concentration that blocked transcription in the presence or absence of kinase inhibitors. Assessment of mRNA decay by Northern analysis (Fig. 3) revealed that all of the cytokine mRNAs induced by virus decayed with a relatively long half-life of several hours. Both H-7 and staurosporine inhibited the virus-induced stabiliza-
Influence of kinase inhibitors on the decay of cytokine mRNAs. (A) Astrocytes were infected with NDV for 6 or 8 h, and then treated with α-amanitin (5 μg/ml) in the presence or absence of the kinase inhibitors H-7 (60 μM), HA1004 (60 μM), or staurosporine (1 μM). Total RNA, collected at the time of α-amanitin addition (t = 0) or after further incubation, was analyzed by Northern blot (10 μg/sample). (B) Summary of cytokine mRNA decay in the presence or absence of kinase inhibitors. mRNA accumulation was quantitated by laser densitometry and standardized to the intensity of β-actin mRNA. α-amanitin alone (○); α-amanitin in the presence of: H-7 (□); staurosporine (▲); HA1004 (▼); and H-7 and CHX (15 μg/ml) (●).

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Address correspondence to Moon Shin, Department of Pathology, University of Maryland School of Medicine, 10 South Pine Street, Baltimore, MD 21201.

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