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Adenovirus infection reverses the antiviral state induced by human interferon

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HeLa cells treated with human lymphoblastoid interferon do not synthesize poliovirus proteins. The antiviral state against poliovirus is reversed if cells are previously infected with adenovirus type 5. A late gene product seems to be involved in this reversion, since no effect is observed at early stages of infection or in the presence of aphidicolin.

Interferon; Adenovirus; Poliovirus; Antiviral state; Herpesvirus

1. INTRODUCTION

Animal cells treated with homologous interferon do not allow the growth of a number of viruses [1]. The molecular basis of the so-called antiviral state is still poorly understood [2,3]. However, a number of new proteins, that appear as a consequence of interferon treatment, can be identified by methods such as 2-D gel electrophoresis [4–6]. Amongst these induced proteins are a 2′-5′-A synthetase and a protein kinase able to phosphorylate eIF2, upon dsRNA stimulation [1–3]. Activation of one of these enzyme activities after viral infection blocks viral translation [1,2]. However, not all animal viruses are equally sensitive to interferon [7]. For example, vaccinia virus grows in several cell lines treated with interferon [7]. Furthermore, translation of vaccinia mRNAs takes place in interferon-treated cells at control levels. Moreover, vaccinia virus infection reverses the antiviral state established by interferon in some cell lines by abolishing its inhibitory effects on translation of several RNA-containing viruses, such as vesicular stomatitis virus and poliovirus [8–11]. This effect is dependent on early gene expression by the vaccinia virus [9,11] and most likely involves inhibition of the IFN-induced dsRNA-dependent protein kinase by an early viral polypeptide [10–13]. The 2′-5′A synthetase activity was reported to be inactivated by vaccinia virus through two viral-mediated enzyme activities: an ATPase that degrades ATP and a phosphatase that dephosphorylates ppp(A2′p)nA [11,14]. In contrast, high levels of 2′-5′A derivatives are found in vaccinia virus-infected cells, irrespective of whether they are pretreated with interferon or not [15]. A complex mixture of authentic 2′-5′A plus non-phosphorylated cores (A2′p)nA and additional non- identified compounds are synthesized in vaccinia virus-infected cells treated with interferon [16]. This mixture activates the 2′-5′A-dependent RNase, at least in cell-free systems [13]. Therefore, the 2′-5′A synthetase and RNase L activities are present in the infected cells, but vaccinia virus can interfere with the antiviral action of interferon by modifying the authentic 2′-5′A. In this manner, the RNase L present in the cell will not be activated by the modified 2′-5′A [16].

The synthesis of proteins in some other virus-infected animal cells is also unaffected by in-

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terferon [7,17–19]. Adenovirus and reovirus infection of HeLa cells treated with interferon are examples [1,19]. Reovirus infection does not reverse the antiviral state when cells are superinfected with VSV or poliovirus [1,17]. However, in this report we show that adenovirus infection does reverse the antiviral state established by interferon and that this reversion is most probably a late function.

2. MATERIALS AND METHODS

2.1. *Cells and virus*

HeLa and Vero cells were grown in culture Petri dishes (Falcon Plastics) containing 10 ml of Dulbecco's modified Eagle's medium (E4D) supplemented with 10% calf serum (Gibco) and incubated at 37°C in a 5% CO_2 atmosphere.

Poliovirus type 1 (Mahoney strain), adenovirus type 5 and vaccinia virus were grown on HeLa cells in E4D medium supplemented with 2% calf serum. Herpes simplex virus type 1 was grown on Vero cells in the same medium. In all cases, the fraction obtained after removal of cell debris by low-speed centrifugation was used as the source of virus. The concentration of virus was estimated by plaque assay.

2.2. *Conditions of infection*

HeLa cells grown in 96-well Linbro dishes were infected with virus at the m.o.i. indicated for each experiment in section 3. After 1 h of incubation at 37°C, the medium was removed and 50 μl of E4D2 were added. The time of virus addition was considered as -1 h and zero time was taken as the point when the virus was removed. Incubation at 37°C was continued until the initiation of radiolabelled protein synthesis.

2.3. *Analysis of proteins by polyacrylamide gel electrophoresis (SDS-PAGE)*

At the times after infection indicated in section 3, 50 μl of methionine-free medium and 1.25 μCi of [^{35}S]methionine (1.45 Ci/mmol; Amersham) were added to the cells which were then incubated for 1 h. The radiolabelled cell monolayers were washed with PBS and dissolved in 50 μl of sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 0.1 M dithiothreitol, 17% glycerol and 0.024% bromophenol blue as indicator). Samples were heated at 90°C for 5 min, and 10 μl were applied to a 15% polyacrylamide gel and run overnight at 100 V/20 cm. Fluorography was carried out with 20% (w/w) 2,5-diphenyloxazole in dimethyl sulfoxide. The gels were dried and exposed as described [20].

Densitometric profiles of the gel were obtained using a Chromoscan 3 (Joyce Loebl) microdensitometer.

2.4. *Interferon*

Human lymphoblastoid interferon Hu-IFN-(Ly) at 1.7×10^6 U/ml was a generous gift from Dr Finter (Wellcome Research Laboratories, England).

3. RESULTS

HeLa cells treated with human lymphoblastoid interferon do not support the growth of poliovirus and, as a result, viral protein synthesis is severely impaired [21]. In agreement with previous results [8,11,12], we found that infection of these cells with vaccinia virus reversed the blockade of poliovirus replication by interferon (fig.1). Therefore, poliovirus protein synthesis occurred to

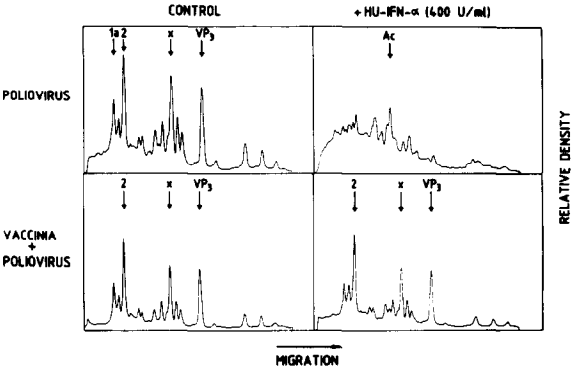

Fig.1. Effect of interferon treatment on protein synthesis in HeLa cells doubly infected with vaccinia and poliovirus. Cells grown in 96-well Linbro dishes were treated with 400 IU/ml of Hu-IFN (Ly) 18 h before vaccinia infection. Poliovirus was added 3 h after infection with vaccinia. Cells were labelled with [^{35}S]methionine and processed as indicated in section 2. The densitometric profiles of the proteins synthesized at 4 h p.i. with poliovirus in single-infected or double-infected cells are shown. Cellular actin (Ac) and polypeptides of viral origin are indicated.

the same extent in vaccinia-infected HeLa cells irrespective of whether they had been pretreated with human lymphoblastoid interferon or not (fig.1). To determine whether this reversion also occurred upon infection with other DNA-containing viruses, HeLa cells treated with interferon were infected with adenovirus type 5 and, after 24 h of infection, they were superinfected with poliovirus. Fig.2 shows that, in agreement with previous work [21], poliovirus protein synthesis was inhibited by interferon, whereas adenovirus proteins were synthesized at control levels. Strikingly, if interferon-treated cells synthesizing adenovirus late proteins were superinfected with poliovirus, the synthesis of poliovirus proteins was observed (fig.2). Similar results were found when the superinfection was carried out with VSV (not shown). To determine whether the expression of an early virus product was responsible for this reversion, HeLa cells, treated with interferon and infected with adenovirus, were superinfected with poliovirus after either 2 or 5 h of adenovirus infection. No reversion was found under these conditions as measured by the appearance of poliovirus proteins (fig.3). Furthermore, adenovirus-infected cells treated with 15 μg/ml aphidicolin, a known inhibitor of DNA polymerase [22,23] became superinfected with poliovirus after 16 h. Again, no reversion was obtained in the presence of aphidicolin (not shown). These results point to an adenovirus late function being responsible for the reversion of the antiviral state. Herpesviruses are able to express their proteins in interferon-treated human cells [18,24,25].

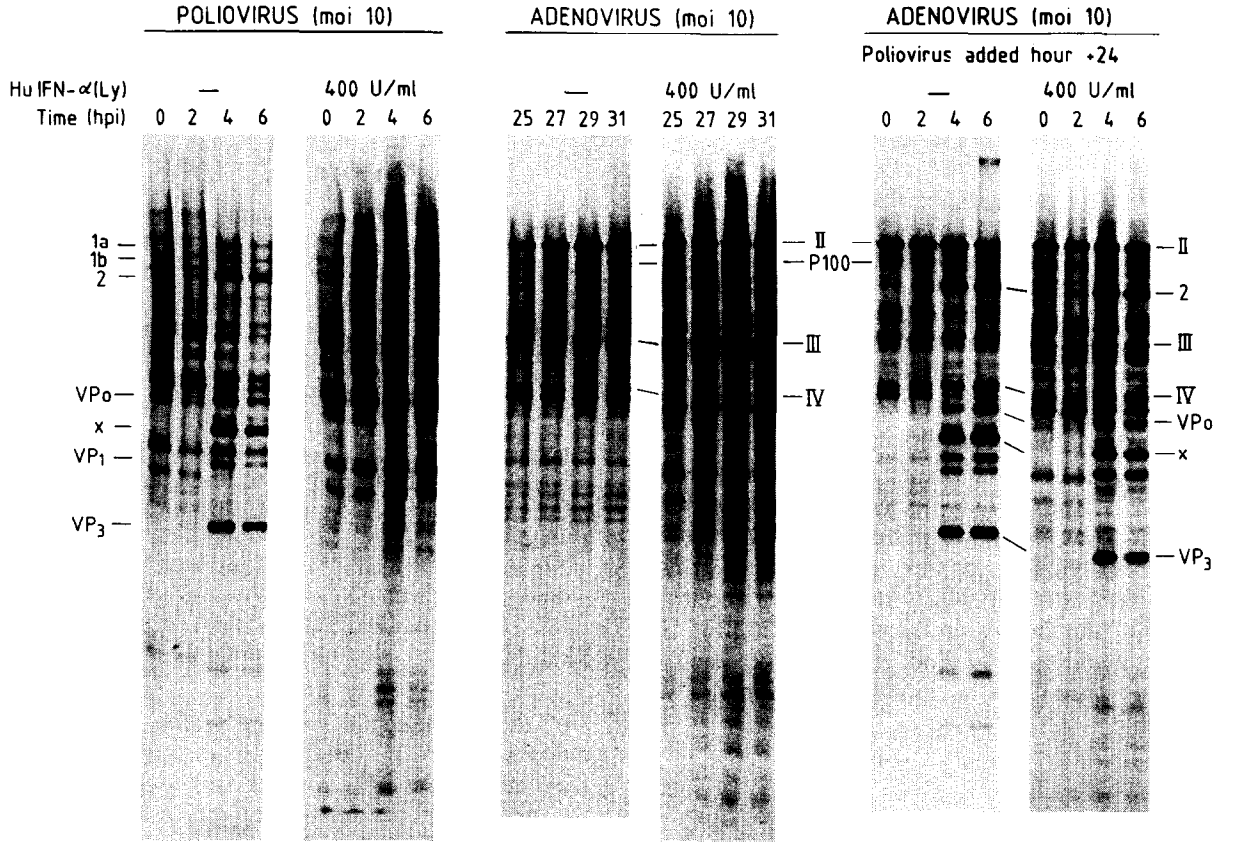

Fig.2. Effect of interferon treatment on protein synthesis in HeLa cells infected with adenovirus and superinfected with poliovirus. Cells grown in 96-well Linbro dishes were treated with 400 IU/ml Hu-IFN- (Ly) 18 h before infection with adenovirus (HPFU/cell). Cells were superinfected with poliovirus 24 h after adenovirus infection. They were then labelled with [^{35}S]methionine and proteins analyzed as described in section 2.

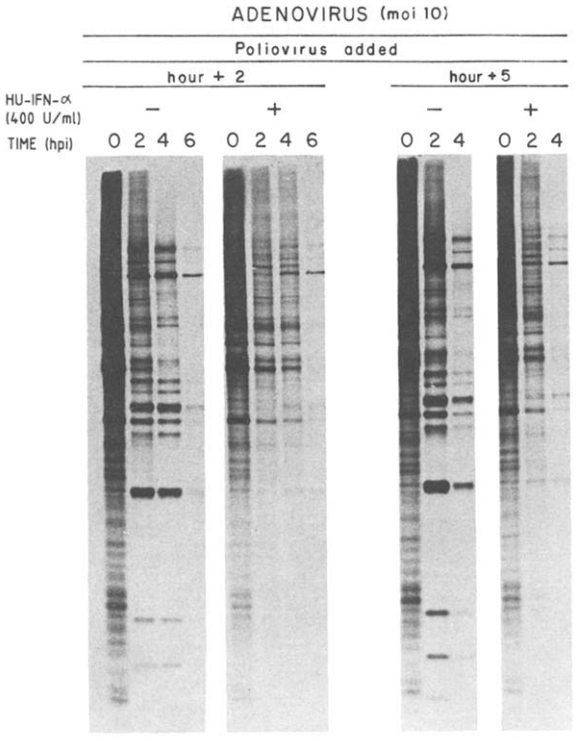

Fig.3. Effect of interferon treatment on protein synthesis in HeLa cells superinfected with poliovirus at early stages of adenovirus infection. Cells grown in 96-well Linbro dishes were treated with IFN 18 h before infection with adenovirus. Cells were superinfected with poliovirus at the indicated times. Proteins were labelled and analyzed as described in section 2.

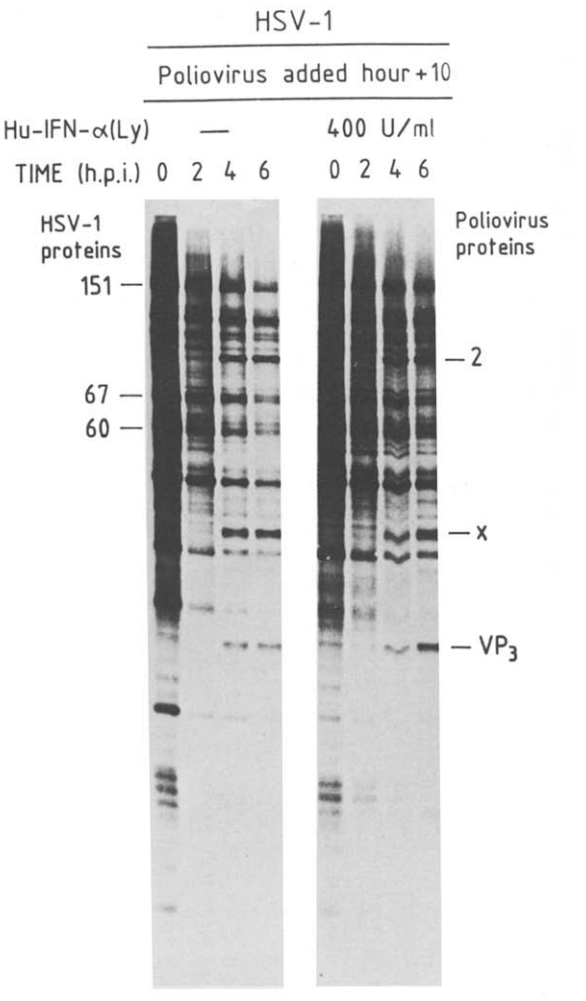

Fig.4. Effect of interferon on protein synthesis in cells infected with HSV-1 and superinfected with poliovirus. Cells were grown in 96-well Linbro dishes and treated with 400 IU/ml of Hu-IFN- (Ly) 12 h before the infection with HSV-1 (5 pfu/cell). They were superinfected with poliovirus (10 pfu/cell) at 10 h after HSV-1 infection. Proteins were labelled and analyzed as described in section 2.

Interestingly, we found that HSV-1 infection also allowed poliovirus proteins to be synthesized in such cells (fig.4). The mechanism(s) triggered by each of these viruses to permit poliovirus protein synthesis remains to be elucidated. Perhaps adenovirus now represents the simplest model to identify the gene products involved in this phenomenon since it produces far fewer proteins than vaccinia and a number of mutants are available.

4. DISCUSSION

The elucidation of the interferon-induced antiviral state at the molecular level may be aided by the availability of new ways to block or reverse it. Since several DNA-containing viruses are able to overcome the antiviral state a detailed knowledge of the mechanisms involved would give new insights into the molecular basis of interferon action against viruses. The mechanisms used by vaccinia virus to avoid the antiviral effects of interferon are mainly based on the inhibition of the DAI-protein kinase [10–13]. Evidence that adenovirus can also block the DAI-protein kinase has accumulated in recent years. Adenoviruses express two small RNAs late in infection known as VA RNAs [26].

VAI RNA is expressed in far larger quantities than VAII RNA. Mutants unable to express VAI RNA grow poorly and do not synthesize late viral proteins in spite of the fact that the mRNAs are present [27]. This translational defect has been correlated with the ability of VAI RNA to block the DAI-protein kinase activity induced after interferon treatment [28,29]. Our findings showing the ability of poliovirus to synthesize proteins in adenovirus-infected cells treated with interferon could be consistent with this idea, since they can be interpreted as an adenovirus blockade of the DAI-protein kinase by means of VAI RNA [29]. However, additional experiments on poliovirus superinfection of VAI RNA(−) mutants in cells treated with interferon would be necessary to confirm the model proposed by these authors. As regards herpesvirus, human cells infected with HSV1 or HSV2 contain high quantities of authentic 2′-5′A, together with other potentially inhibitory derivatives [24]. The synthesis of these compounds which interfere with the 2′-5′A-dependent RNase, may explain the reversion we have found. The situation for herpesviruses will be similar to that for vaccinia virus, i.e. the RNase L present in the cell will not become activated by the virus-modified 2′-5′A [24].

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