The Left Part of the Viral Genome Is Sufficient for Interferon Induction by Adenovirus Type 12

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With 5 Figures

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Summary

Adenovirus type 12 is a potent interferon inducer on chick embryoio cells. Incomplete particles induce similar levels of interferon as complete virions, even if they contain only the left 20 per cent of the genome. Empty capsids lacking DNA are not able to induce interferon, suggesting that part of the viral genome is required to trigger the cells to produce interferon.

Introduction

Interferons (IFNs) are inducible proteins with pleiotropic biological activity (antiviral, anticellular, immunomodulating, etc.), which are produced by cells in response to a number of inducers (viruses, bacteria, toxins, nucleic acids, etc.). Most viruses trigger cells to produce IFN, including human adenoviruses (1, 2). Adenoviruses are not able to induce IFN in human cells, but produce IFN in vitro in chick cells and in vivo in chickens (9).

As our recent findings (13) have proved, human adenovirus serotypes can be divided into two groups on the basis of their IFN-inducing ability (potent and weak inducers). The group of potent inducers consists of types Ad8, Ad12, Ad18, and Ad31, inducing high titres of IFN (4—6x10³ international units (IU) per ml when optimum virus doses are used). Weak inducers, Ad2, Ad3, Ad4, Ad5, Ad6, Ad7, Ad15 and Ad19, induce IFN of low titre (about 100 IU per ml) even if optimum virus doses are used. The IFN-inducing ability of potent inducers can be abolished by UV irradiation, suggesting a role for functional viral DNA in IFN induction. In contrast,
the low IFN-inducing capacity of the nonpotent inducers is extremely UV-resistant (13).

In the present paper we report further evidence that the IFN-inducing component and probably the induction mechanism of Ad12 (a potent inducer) and Ad2 (a weak inducer) are different.

Materials and Methods

Cells and Viruses

Adenovirus types 2 and 12 were propagated in HEp-2 monolayer cells or in suspension cultures of KB cells. Secondary chick embryo cells (CEC) were used for production and assay of IFN. Vesicular stomatitis virus (VSV) Indiana strain was used as challenge virus to evaluate the titre of IFN.

Separation of Incomplete Particles (IP) and Empty Capsids (EC)

To obtain intact IP and EC from Ad2 pools, a previously described purification method was used which involves several CsCl density gradient centrifugations in 5 per cent glycerol (12). Briefly, the adenovirus-infected cells were homogenized in a sonicator, and the suspension was extracted with trichlorotrifluoroethane and layered onto the top of a discontinuous density gradient consisting of two CsCl layers (densities of 1.5 and 1.2 g/ml). The gradients were centrifuged for 3 hours at 25,000 rev/min in a Beckman SW27 rotor. The IP and EC were further separated in a second step density gradient composed of three CsCl layers (densities of 1.4, 1.3 and 1.2 g/ml) by centrifugation at 25,000 rev/min for 12 hours in an SW41 rotor. Equilibrium centrifugation of pooled IC and E particles was performed twice in separated tubes. All CsCl solutions contained 5 per cent glycerol. The tubes containing IP were punctured, and equal fractions (5 for Ad12 and 7 for Ad2) from the virus-containing part of the gradient were collected, representing different classes of IP. From other tubes, highly purified complete and empty particles were obtained. The purity and integrity of the IP and EC were controlled by electron microscopy as described (12). Particles were not aggregated and the proportion of collapsed and damaged particles was less than 3 per cent.

Interferon Induction and Interferon Assay

IFN induction in CEC was performed as described previously (1). IFN was titrated by a micromethod monitoring the protection of CEC from the cytopathic effect of VSV (13). IFN titres were expressed in IU per ml, compared to a reference standard chick IFN preparation (MRC Research Standard A, 62/4, Mill Hill, London).

Gel Electrophoresis, Blotting and Hybridization

DNA samples from Ad12 complete virions (CV) and IP or BglII and HindIII endonuclease (Bethesda Research Laboratory) cleaved Ad12 DNA were electrophoresed in 1 per cent agarose gela. The DNA was isolated with phenol-chloroform extraction after disrupting and digestion of particles with 1 per cent SDS and 100 µg/ml proteinase K (Merck).

The restriction endonuclease digestions were carried out according to the manufacturer's directions at 2 enzyme units per µg DNA for 2 hours.

The DNA was blotted onto nitrocellulose as described (10). Prehybridization and hybridization were carried out according to WAHL et al. (15).

DNA samples from complete and incomplete particles were labelled with 32P by nick-translation to specific activities of $1-2 \times 10^6$ cpm/µg DNA.