Interferon induction by adenoviruses

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Summary

All human, simian, bovine and avian adenovirus types tested so far and the canine hepatitis virus induce interferon production in chick cells. This finding indicated this property to be characteristic for viruses belonging to the adenovirus group. Trypsin treatment, which had no effect upon the infectivity, diminished or eliminated the interferon-inducing abilities of crude adenoviruses, and thus the need for a trypsin-sensitive protein in interferon induction was suggested. T antigen and interferon were formed simultaneously in chick embryo fibroblast cells infected with human adenovirus type 12, and therefore the adenovirus-specific T antigen was resistant to the action of endogenous interferon synthesized by the same cells. In chicks inoculated with human types, the appearance of interferon was biphasic: an 'early' and a 'late' interferon could be demonstrated with maximum titre 4 and 10 hr, respectively, after virus infection. In chicks infected with adenoviruses, first interferon production and then a decreased primary immune response to sheep red blood cells was observed. It was assumed that in adenovirus-infected chicks the interferon produced by viral stimulus resulted in a transient immunosuppression.

Introduction

Since the discovery by Isaacs and Lindenman (1957) of interferon induction by influenza virus in chick cells, it has been demonstrated that nearly all known animal viruses, either DNA or RNA, induce interferon, and living cells of different animal origins produce interferon as their response to viruses, other agents or materials (Vilcek, 1969; Ho, 1973).

In 1967 it was observed (Béládi and Pusztai, 1967) that chick embryo fibroblast cells inoculated with

human adenoviruses produced interferon. Other cells tested, such as human, simian, canine, murine and hamster cells, did not synthesize interferon upon infection with human adenoviruses. The present paper presents results obtained in adenovirus-infected chick cells both *in vitro* and *in vivo*.

Materials and methods

The adenoviruses were grown in cells of their host species, i.e. canine hepatitis virus in primary dog kidney cells, bovine adenovirus in primary bovine kidney cells, simian adenoviruses in secondary rhesus monkey kidney cells, GAL and CELO viruses in primary kidney cells of 6-day-old chickens, and human adenoviruses in HEP-2 cells. Sindbis virus was propagated in chick embryo fibroblast cells.

In some experiments, semi-purified (Arcton-treated virus) and purified adenoviruses were used. The method employed for purification was described earlier (Taródi *et al.*, 1977).

The infectivity of an adenovirus was usually determined in tube cultures with the dilution method. The median tissue culture dose (TCD₅₀) was calculated using the formula of Reed and Muench (1938). Plaque assay (Williams, 1970) was used for quantitation of the infectivity of some human types. To evaluate the infectivity of Sindbis virus, the plaque method was employed (Béládi and Pusztai, 1967).

The assay of interferon was performed in chick embryo fibroblast monolayers by the plaque reduction method (Béládi and Pusztai, 1967).

An indirect immunofluorescence technique (Pusztai, Szabó and Béládi, 1977) was used to examine the appearance of T antigen in chick embryo fibroblast cells infected with adenovirus type 12.

For *in vivo* experiments, 4- to 8-week-old chicks obtained from different local suppliers were used. The viruses were injected intravenously into the

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chicks. After inoculation, blood samples were collected at different intervals for determination of their interferon content.

The number of 19S haemolytic plaque-forming cells (HPFC) in the spleen of chicks immunized with sheep red blood cells (SRBC) was determined by using the Jerne technique in gel as modified by Dresser and Wortis (1967). White cells were counted in a haemocytometer by using Natt-Herrick solution (Natt and Herrick, 1952) as diluent. The trypan blue exclusion procedure was used as the criterion for cell viability. Direct antibody plaques detected after incubation with complement were regarded as being due to cells releasing 19S immunoglobulin M haemolysins, and the total number of HPFC per 10^6 spleen cells was then calculated.

For immunization, 1.2×10^9 SRBC/100 g body weight were inoculated intravenously into chicks. The method used for electron microscopy is described elsewhere (Béládi *et al.*, 1974). The pictures were taken with a JEOLCO JEM type 100B electron microscope, with the use of 80 kV and an objective aperture of 40 μ .

Results

Until now, 12 different human types have been studied for interferon induction in chick embryo fibroblast cells. Each type induced interferon and the titres obtained ranged from 8 to 1024 (Table 1). Leucocytes from 4- to 8-week-old chicks, cultivated *in vitro*, regularly synthesized a higher amount of interferon after adenovirus infection than chick embryo fibroblast cells (Table 2).

It was observed that trypsin treatment reduced or eliminated the ability of crude human adenoviruses to induce interferon in chick embryo fibroblast cells (Table 3). At the same time, treatment with trypsin did not alter their infectivity in HEP-2 cells.

TABLE 1. Interferon induction by human adenoviruses in chick embryo fibroblast cells

Virus			
Subgroup	Type	Dose*	Interferon titre
1	3	5.5	128
	16	6.5	64
2	4	4.5	64
	8	2.5	256
	10	3.7	32
	19	3.7	8
	27	4.2	128
3	2	7.5	32
	5	8.0	64
	6	8.5	64
4	12	4.5	1024
	18	6.5	32

* log TCD₅₀.

TABLE 2. Interferon induction by human adenoviruses in chick leukocytes

Virus			Interferon titre
Subgroup	Type	Dose*	
1	14	6.3	128
	16	5.3	128
2	8	1.8	256
3	5	8.3	256
	6	8.8	64
4	12	4.8	4096

* log TCD₅₀.

TABLE 3. Effect of trypsin on the interferon-inducing ability of human adenoviruses

Virus		Titre of interferon induced by:	
Subgroup	Type	Untreated virus	Trypsin-treated virus
1	3	16	< 4
2	4	256	4
3	5	64	< 4
	6	16	< 4
4	12	1024	4

The interferon-inducing abilities of types 6, 8 and 12 at different multiplicities of infection were compared in chick embryo fibroblast cells. It was found that about 1000 times smaller TCD₅₀ of type 12 and about 10 000 times smaller TCD₅₀ of type 8 induced 64 times more interferon than did type 6 (Table 4).

TABLE 4. Interferon induction by human adenovirus types 6, 8 and 12 at different multiplicities

Virus			Interferon titre
Type	Dose (log TCD ₅₀)	Multiplicity (log)	
6	7.5	0.32	128
	6.5	-0.68	4
	5.5	-1.68	< 4
	4.5	-2.68	< 4
8	2.5	-4.68	256
	1.5	-5.68	32
	0.5	-6.68	8
	0.05	-7.68	< 4
12	4.5	-2.68	1024
	3.8	-3.38	512
	3.5	-3.68	256
	2.5	-4.68	32

The interferon inductions by types 8 and 12 heated at 56°C were tested. After heating for different periods, both the infectivities and the interferon-inducing capacities were reduced (Fig. 1). In contrast, interferon induction was much less sensitive

FIG. 1. Effect of heating at 56°C on the infectivity and interferon-inducing ability of adenovirus types 8 and 12. Type 8; ●▲ infectivity; — Type 12; ○△ interferon.

FIG. 2. Effect of u.v. irradiation on the infectivity and interferon-inducing ability of adenovirus types 8 and 12. Type 8; — Type 12; ●▲ infectivity; ○△ interferon.

to u.v. irradiation than the infectivity, i.e. at u.v. doses which completely eliminated the infectivity, a considerable capacity to induce interferon remained (Fig. 2).

Human types purified by CsCl density gradient centrifugation also induced interferon, but the titres of interferon were not higher than that induced by the crude virus (Table 5).

The possible correlation between the formation of paracrystalline structures during replication in HEP-2 cells and the induction of interferon in chick embryo fibroblast cells by human adenoviruses was studied. For this purpose types 6, 8, 12 and 16 were

tested, of these types 6 and 16 were less effective inducers. Paracrystalline structures were found only in HEP-2 cells infected with type 6 (Fig. 3). Type III inclusions could be observed in cells infected with types 8 and 12 (method of Martinez-Palomo, LeBiu and Bernhard, 1967) (Fig. 4).

TABLE 5. Interferon induction by purified adenoviruses in chick embryo fibroblast cells

Virus		Interferon titre
Type	Dose (PFU)	
5	4.5×10^9	64
6	1.5×10^9	128
12	3.1×10^6 *	1024

* TCD_{50} .

Like the human types, the simian, bovine, avian and canine adenoviruses induced interferon in chick embryo fibroblast cells (Table 6).

TABLE 6. Interferon production in chick embryo fibroblast cells inoculated with adenoviruses of different origin

Adenovirus	Dose*	Interferon titre
Human type 12	4.5	1024
Bovine type 2	5.5	64
Canine hepatitis	6.5	64
GAL	5.0	512
CELO	5.5	64
Simian type 15	6.5	32
Simian type 17	5.5	128

* $\log \text{TCD}_{50}$.

The formation of both T antigen and interferon in chick embryo fibroblast cells infected with human type 12 was demonstrated. The appearance of T antigen 30 hr after infection is shown in Fig. 5. It can be seen that the syntheses of T antigen and interferon were closely dependent on the multiplicity of infection in the chick cells (Table 7).

TABLE 7. T antigen and interferon production in chick cells infected with adenovirus type 12*

Virus dose ($\log \text{TCD}_{50}$)	T antigen-positive cells (%)	Interferon titre
6.65	100	1024
5.95	30	512
5.65	15	256
4.65	1	32

* Semi-purified virus.

The *in vivo* interferon-inducing abilities of human adenoviruses were also tested. The appearance of interferon proved to be biphasic, i.e. 'early' and 'late' interferon could be observed, in the sera of

FIG. 3. Electron micrograph of HEP-2 cell 72 hr after infection with type 6. Intranuclear paracrystals are present. ($\times 2250$).

FIG. 4. Electron micrograph of type 12 inoculated HEP-2 cell 72 hr after infection. Type III inclusions (arrows) are in the nucleus. ($\times 10\,050$).

chicks inoculated with type 12 (Fig. 6). Chicks inoculated with trypsin-treated type 12 showed that, whereas the formation of 'early' interferon was eliminated, there was no effect upon the 'late' interferon production. Purified adenoviruses induced higher amounts of interferon in chicks than did crude viruses (Table 8).

It was observed that types 6 and 12 were weak antigens in chicks, and therefore the primary immune response to SRBC of adenovirus-infected chicks was investigated. Chicks given an SRBC stimulus within the first 48 hr of adenovirus infection responded with numbers of 19S HPFC in the spleen comparable to those obtained in uninfected birds.

FIG. 5. Chick embryo fibroblast cells containing adenovirus 12 T antigen in their nuclei and cytoplasm. ($\times 500$.)

TABLE 8. Interferon induction by crude and purified adenovirus type 6 in chicks

Virus dose (log TCD ₅₀)	Titre of interferon induced by virus	
	Crude	Purified
7.5	8	—
9.2	16	—
9.7	—	128
10.5	—	512

FIG. 6. Interferon production in chicks inoculated with human adenovirus type 12. ●—● Untreated virus; ○—○ trypsin-treated virus.

FIG. 7. Interferon production and immune response to SRBC in adenovirus type 12 infected chicks. ○—○ Control; ○---○ virus-infected; ▲—▲ interferon.

Marked decreases were observed when SRBC were given 4–7 days after virus injection (Fig. 7). On the other hand, interferon production took place earlier, the peak of serum interferon being found on the first day after virus infection; its amount then decreased rapidly.

Discussion

Adenoviruses of different origins induce interferon in chick embryo fibroblast cells (Béladi *et al.*, 1970), and thus this property might be assumed as being generally characteristic of adenoviruses.

Differences in degree of inducing ability among human types have been observed both in chick embryo fibroblast cells (Pusztai *et al.*, 1969b) and in chick leucocytes (Mucsi *et al.*, 1970). Types 8 and 12 proved to be the most effective inducers, and the data revealed that it was not the infectivity of the virus which was important for interferon induction. Trypsin treatment of the crude virus reduced or eliminated the interferon-inducing capacity of human types; this suggested the role of a trypsin-sensitive protein in the interferon induction. However, this observation was not confirmed when purified virus was used (Taródi *et al.*, 1977). The reason for this difference remains to be answered.

The fact that, considering their infective titres, purified human types were less effective inducers *in vitro* than crude ones, together with the differences observed in the interferon-inducing abilities of different human types, suggested the possibility that paracrystalline structures in some of the crude virus materials might be responsible for the different activities. On the basis of the fact that paracrystalline structures were observed only in the case of type 6, which is not an effective inducer, it is very likely that the effectiveness of an adenovirus strain as an inducer does not depend on its paracrystalline structure-forming ability. The effective inducers stimulated the formation of type III inclusions. These inclusions were described as consisting of DNA and protein (Martinez-Palomo *et al.*, 1967). The significance of these inclusions with respect to the interferon induction by adenovirus types 8 and 12 remains to be elucidated.

Heating at 56°C reduced the infectivity and the interferon-inducing ability of types 8 and 12 in parallel, while the effect of u.v. irradiation was more pronounced upon the infectivity than on the interferon-inducing capacity. These data supported the assumption that it is not the infectivity of the adenovirus which plays the decisive role in interferon induction.

Differences have also been observed between the *in vitro* and *in vivo* interferon-inducing capacities of adenoviruses. Trypsin treatment eliminated the *in vitro* interferon-inducing ability of the crude virus; however, it had no effect upon the late interferon-inducing capacity of that virus *in vivo* (Pusztai *et al.*, 1969a). In contrast to the *in vitro* observation, purified adenoviruses were more effective inducers *in vivo* than unpurified ones. One possible explanation for this might be that a different determinant (or determinants) of the virus plays a role in the interferon induction *in vitro* from that *in vivo*. On the other hand, one may assume that the reason for the differences is the different cell types involved in *in vitro* and *in vivo* interferon production.

Human adenoviruses are not pathogenic for a

variety of common laboratory animals, but marked antibody responses have been noted in guinea-pigs, cotton-rats, rabbits and hamsters after a single inoculation with different types (Rowe *et al.*, 1955). Adenovirus-infected chicks showed no gross pathological alterations, and no marked change in the peripheral leucocyte counts was found. Consistently, chicks infected with adenoviruses had similar spleen weights to those of the control birds. Spleen cell viability studies showed no differences between infected and control birds (Béládi *et al.*, 1973). In adenovirus-infected chicks, first, interferon production and, later, a decreased response to SRBC only could be demonstrated. The peak of serum interferon was observed on the first day after infection; the amount of interferon then decreased, and no interferon was detectable on the fourth day following virus inoculation. However, immunosuppression was not found when SRBC was inoculated on the first or second day after virus infection; SRBC given together with type 12 adenovirus slightly increased the number of HPFC. Marked immunosuppression was observed when the inoculation of SRBC took place 3 or 7 days after virus infection, and this state lasted for a further 10–14 days (Béládi *et al.*, 1973). These data suggested a correlation between the interferon production and the transient immunosuppression induced by human adenoviruses in chicks.

Interferon has been shown to inhibit the antibody response to T cell-dependent and independent antigens both *in vitro* (Gisler, Lindhal and Gresser, 1974; Johnson, Smith and Baron, 1975; Johnson, Bukovic and Baron, 1975; Booth *et al.*, 1976) and *in vivo* (Braun and Levy, 1972; Chester, Paucker and Merigan, 1973; Brodeur and Merigan, 1974; Hirsch *et al.*, 1974; De Maeyer, De Maeyer-Guignard and Vandeputte, 1975), when inoculated before or at the time of antigen stimulation. On the basis of these data, it is considered that the interferon produced in adenovirus-infected chicks is responsible for the transient immunosuppression demonstrated.

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