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Induction by Sendai Virus of Non-transmissible Cytopathic Changes Associated with Rapid and Marked Production of Interferon.* (26921)

ION GRESSER** (Introduced by J. F. Enders)

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During experiments with various myxoviruses it was noted that high concentrations of chick adapted Sendai virus induced non-transmissible cytopathic effects (NTCE) in cultures of primary human amnion (HA) cells. Several observations suggested that Sendai virus underwent an incomplete cycle of multiplication in these cells, which was associated with the early liberation of large quantities of an interferon-like substance into the culture medium. These observations are described together with experiments suggesting that these phenomena may be interdependent.

Methods and materials. Cell culture. Preparation and maintenance of human amnion (HA) cells has been previously described (1). Primary cultures of rhesus monkey kidney cells were obtained commercially.†

Viruses. Relevant data on viruses employed are as follows:

Sendai virus (F. Davenport) allantois of embryonated egg 27

Sindbis virus (R. Taylor Ar-339) HA cells 10

Poliovirus II MEF chick embryo cell cultures 143, HA cells 2

Mumps virus HA cells 7

Simian virus 5, monkey kidney cells 4

DA virus‡ (P. Isaacson Rh 76) HA cells 3

Preparation of stock sendai virus. Sendai virus was prepared in 9-11 day embryonated eggs by inoculation intrallantoically of 0.1 ml of a 10^{-3} dilution of stock virus. After 72 hours incubation at 35-37°C, allantoic fluid was harvested, centrifuged for 10 minutes at 400 g, and the supernate centrifuged at 4°C for 2 hours at 110,000 g (Spinco L preparative centrifuge #40 rotor). The sediment was resuspended in a mixture of 50% tissue culture medium and 50% skimmed milk and stored at −70°C.

Virus assay. Infectivity titers were calculated by the formula of Reed and Muench. Sendai virus was titrated in embryonated eggs (EID_{50}) taking as criterion of infection appearance of hemagglutinin in allantoic fluids. Sindbis, poliovirus, DA, SV 5 and mumps viruses were prepared and titrated in HA cells. In this system more than 25% cell destruction was considered evidence of viral multiplication or NTCE in the case of Sendai virus. Hemagglutination and hemadsorption tests were performed by standard methods(2,3).

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‡ A myxovirus isolated from the blood of a patient with viral hepatitis. This virus is considered closely related if not identical to SV 5 and SA viruses.

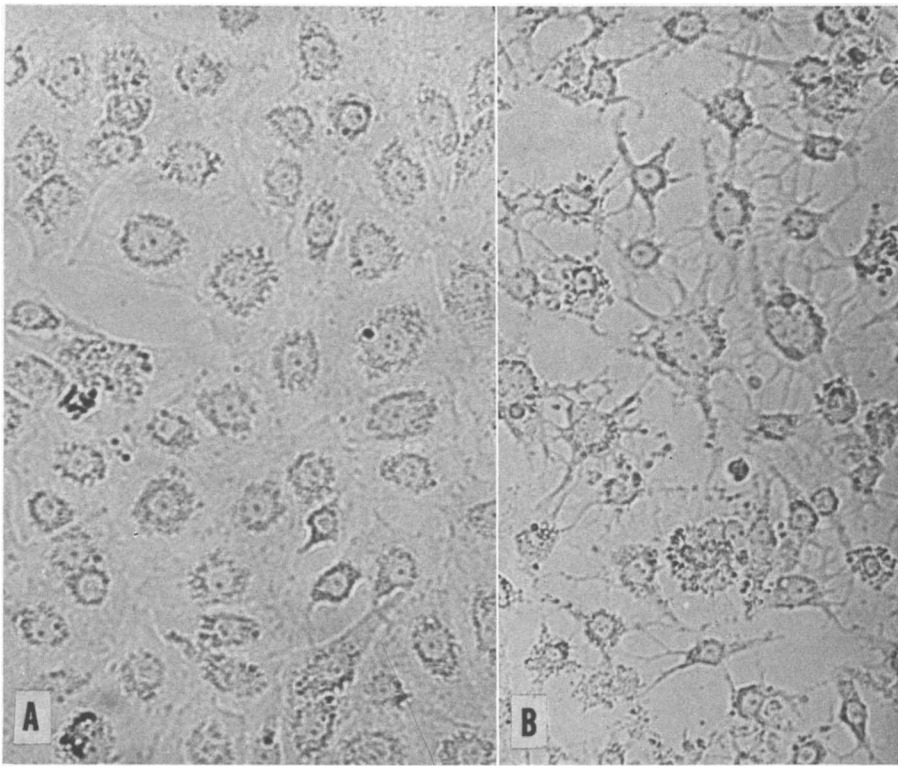

FIG. 1. A. Uninoculated human amnion cells unstained. B. Human amnion cells 24 hr after inoculation of chick-adapted Sendai virus unstained. Mag. × 350.

Antisera. Anti-Sendai rabbit serum was obtained through the courtesy of Dr. J. Lewis, the Sterling Winthrop Co., Rensselaer, N. Y.

Assay of interferon. All preparations of interferon were centrifuged at 110,000 g at 4°C for 2 hours. The supernate (interferon) was free of infectious virus and hemagglutinin, and in some instances anti-Sendai rabbit serum in excess was added. Interferon was assayed in primary tube cultures of HA cells previously incubated in stationary racks at 35-37°C for 4 or more weeks. Two-fold dilutions of interferon (2-3 tubes/dilution) were incubated with cells for 18-24 hours prior to inoculation of 100 $TCID_{50}$ of Sindbis virus. Complete destruction of cells in control cultures regularly occurred in 3 days. Interferon titer was expressed as the highest dilution which protected more than 75% of the cell sheet in at least one of 2 cultures for at least 3 days after complete destruction of cells had occurred in control cultures. Cultures exhibiting more than 25% CPE were regarded as interferon-negative although marked differences from control cultures in time of onset and extent of CPE were often observed.

Results. Morphologic changes. Infected (Sendai virus) allantoic fluid in appropriate dilutions added to cultures of HA cells induced diffuse and marked NTCE. This consisted of shrinkage and loss of cytoplasm, appearance of elongated cytoplasmic process and relative prominence of the nucleus (Fig. 1). NTCE was first noted between 6-12 hours after addition of the agent, depending upon its concentration, and was complete between 24-48 hours. Thereafter affected cells gradually disappeared.

Association of NTCE with infectious virus. These changes were not elicited after inoculation of a) control allantoic fluid, b) ultraviolet irradiated infected allantoic fluid, c) supernate of ultra-centrifuged infected allantoic fluid, or d) aliquots of a previously incubated mixture of Sendai virus and antiserum.

Non-transmissibility of cytopathic effect. From Table I it can be seen that the cyto-

TABLE I. Failure to Transmit Cytopathic Effect Serially in Human Amnion Cells.

Virus dilution	Passage 1	2	3
1:10	+	±	0
1:100	+	0	0
1:1000	±	0	0
1:10,000	0	0	0

+, CPE; ±, partial CPE; 0, absence of CPE.

pathic effect described was not transmissible to uninoculated cultures. At a viral dilution of 1:10 some effect was still noted on second passage. Since on the third passage no changes were observed, it seems evident that this effect in the 2nd passage was due to residual virus derived from the original inoculum. This CPE was not observed on second passage if the original inoculum was removed several hours after exposure to HA cells and the cell sheet washed prior to replacement of maintenance medium.

To induce NTCE in HA cells, large amounts of virus (1-10 EID_{50} per cell) were required, thus bringing the ratio of EID_{50} to TCD_{50} to about 10^{5-6} :1. No multiplication of infectious virus was demonstrated in HA cells after inoculation of large amounts of virus. The presence of 50,000 EID_{50}/ml of virus at 3 hours (Table II) may be attributed to residual virus after removal of the original inoculum and washing of the cell sheet at 1 hour. Forty-eight hours after inoculation, the titer of virus had decreased to 16,000 ID_{50}/ml. With a small inoculum, (*i.e.*, 1000 EID_{50}) of virus, slight multiplication of infectious virus did occur. Thus after removal of the original inoculum and washing of the cell sheet prior to introduction of fresh medium, no infectious virus was detected at 3 hours, and 320 EID_{50}/0.1 ml of virus was present 48 hours after inoculation.

If guinea pig erythrocytes were added just prior to or upon the first indication of NTCE, focal areas of hemadsorption were observed. At the time of maximum NTCE the cell sheet was obscured by adsorbed erythrocytes and hemagglutinin in low concentrations (dilutions of 1:2-1:8) was demonstrable in culture fluid (Table II).

Though other cell virus systems were not extensively investigated, chick-adapted Sendai virus was found to induce NTCE in human kidney, WS and HeLa cells. Monkey kidney cells appeared unaffected by Sendai virus though hemadsorption of guinea pig erythrocytes was demonstrated 2 days after inoculation.

Interferon production. After infection of HA cells with ultra-centrifuged chick-adapted Sendai virus, a substance possessing certain properties characteristic of interferon appeared in the culture medium (Table III). In the viral inoculum itself no interferon was detected. Significant quantities of interferon were found 12 hours after inoculation and maximum titers were attained at 24-48 hours (Table II). The amount of interferon produced was roughly proportional to the concentration of virus in the original inoculum (Table IV). Titers ranging between 1:256 to 1:1024 were consistently recorded.

Species specificity of interferon. High concentrations of Sendai interferon (1:1024) produced in HA cell cultures did not protect chick embryo cells against challenge with 50 $TCID_{50}$ of Sindbis virus.

Interferon prepared in monkey kidney cells

TABLE II. Effects of Sendai Virus on HA Cells.

Time (hr)	1.5*	3	6	12	24	48
CPE	0	0	Trace	50%	100%	100%
Hemadsorption	0	0	Trace	Marked		
Hemagglutinin (uncentrifuged medium)	<1:2	<1:2	<1:2	<2	1:2	1:8
Egg infectious virus log ID_{50}/ml	—	4.7	—	—	—	4.2
Titer of interferon produced (ultracentrifuged supernate)	<1:4	1:6	1:6	1:96	1:768	1:768

* Original inoculum $10^{8.3}$ EID/ml was removed after 1 hr incubation at 37°C and the cell sheet washed several times with Hanks' salt solution before replacement of maintenance medium.

TABLE III. Properties of Interferon Liberated by HA Cells after Infection with Sendai Virus.

Procedure	Result
Incubation with crystalline	
Trypsin, 0.5 mg/ml, 37°C 1 hr	Complete loss
DNA-ase, *idem*	No effect
RNA-ase, "	" "
Heat 60°, 1 hr pH 7.8	Complete loss
20% ether, 18 hr 4°C	4-8 fold decrease
pH 4, dialysis, 24 hr 4°C	No effect
Storage 4°C–20°C, 2 mo	" "
Incubation with Sendai antiserum	" "
Ultracentrifugation 110,000 g, 2 hr 4°C	" "

by infection with Sendai virus was effective at a dilution of 1:512 in protecting HA cells against challenge with 100 $TCID_{50}$ of Sindbis virus, but did not protect chick embryo cells at a dilution of 1:4.

Relative lack of effectiveness of interferon against NTCE. Interferons produced by HA cells after infection with either Sendai or measles virus were effective at dilutions of 1:1024 (Sendai) or 1:256 (measles) in protecting HA cells against challenge with a 100 $TCID_{50}$ of Sindbis or polio viruses. The same interferon preparations, however, were far less effective in preventing the NTCE in HA cells induced by Sendai virus. Thus with interferon dilutions of 1:2 only a 16-fold decrease in NTCE titer was observed, and at interferon dilutions of 1:4 and 1:8 the difference in NTCE between treated and untreated cells was much less (4- and 2-fold respectively).

Human amnion cells were infected with mumps, SV_5 or DA viruses, and challenged with Sendai virus 4-6 days later when cytopathic effect due to these agents was minimal. No NTCE was observed even after introduction of 10^8 EID_{50} of Sendai virus. This protection afforded by previous infection of cells contrasted with the relative ineffectiveness of stock interferon to repress significantly Sendai NTCE. Moreover this effective inhibition could not be attributable to the presence of virus or interferon in the medium nourishing infected cells, since it was undiminished after removal of medium and washing of the cell sheet prior to challenge. When appropriate antisera (anti-mumps, anti-SV5 etc.) were added to cultures previously infected with these agents before challenge with Sendai virus no hemadsorption occurred, though in challenged uninfected control cultures NTCE and hemadsorption were noted. Absence of Sendai NTCE in cultures previously infected with the other agents was therefore confirmed by the absence of hemadsorption. Accordingly one may infer that previous infection of cells with these viruses either prevented entry of Sendai virus into the cell or prevented viral replication and NTCE.

Discussion. The non-transmissible cytopathic effect in HA cells occurring several hours after addition of high concentrations of infectious chick-adapted Sendai virus resembles that observed with other myxoviruses in various cell cultures(4). The yield of hemagglutinin was in most instances low and that found may possibly represent release of hemagglutinin adsorbed from the original inoculum. Even if this were true, however, the appearance of hemadsorption 6 hours after inoculation, concomitant with onset of NTCE and failure to demonstrate an increase in infectious virus strongly suggest an incomplete cycle of viral multiplication. Whether cellular destruction results from cytoplasmic replication of viral components that remain non-infectious or whether it results from a mere accumulation from the inoculum of a large number of infectious viral particles within the cell remains undetermined.

The similarities between the viral inhibitory substance present in the medium of Sendai infected HA cells and previously described interferons would seem to justify application of the term interferon to this factor. It must be emphasized that significant levels of interferon were attained as early as 12

TABLE IV. Effect of Dilutions of Sendai Virus on Production of Interferon.

Dilution of virus*	CPE (48 hr) %	Interferon titer (48 hr)
10^{-2}	100	1024
10^{-3}	100	384
10^{-4}	100	384
10^{-5}	0	64
10^{-6}	0	12

* Egg infectivity titer 10 $EID_{50}/10^{0.3}$ $EID_{50}/0.1$ ml.

hours after inoculation. The rapid rate of interferon production and high titers obtained may be idiosyncratic of this virus. On the other hand it is tempting to speculate that the "success" of interferon production is related to the failure of HA cells to support multiplication of infectious virus. Whether the early appearance of interferon is responsible itself for this abortive cycle of multiplication remains speculative. Some support for this latter hypothesis may be found in the demonstration of slight multiplication of infectious virus in HA cells after inoculation of a relatively small concentration of virus.

Henle and coworkers(5) noted that Sendai virus underwent an incomplete cycle of replication in MCN cells. To assay the effect of various interferons on Sendai virus infection in these cells, they determined the titer of hemagglutinin liberated after challenging control cultures and cultures pretreated with undiluted interferon. A decrease in hemagglutinin titer of 8-100 times was observed after preincubation of cells with interferon for 24 hours. These results are comparable to the 16-fold decrease in NTCE titer observed in our experiments after incubation of HA cells with undiluted or very low dilutions of interferon. This same interferon preparation, however, when diluted several hundred fold completely inhibited CPE after inoculation of over a 100 $TCID_{50}$ of Sindbis or polio virus. If Sendai NTCE can eventually be shown to depend upon the cytoplasmic incomplete cycle of viral synthesis our results would afford support for Isaac's contention that interferon is less effective in suppressing viral components in the cytoplasm than it is in the nucleus(6).

To follow this line of speculation further it is possible that interferon may play a decisive role in the well-known increase of the hemagglutinin/infective virus ratio that follows inoculation of high concentrations of certain viruses (*i.e.*, influenza virus). Thus in serially passing high concentrations of influenza virus, one may well also transfer interferon. If cytoplasmic viral reproduction is less affected by interferon, the production of hemagglutininative virus would be less inhibited by interferon than that of complete virus.

It may be pointed out that the contrast between the marked resistance of cells previously infected with other viruses to NTCE of Sendai virus and that conferred by interferon indicates that the mechanisms of the resistance may well be different.

Summary. High concentrations of chick-adapted Sendai virus induced non-transmissible cytopathic effects in cultures of primary human amnion (HA) cells. Evidence was presented suggesting that an incomplete cycle of Sendai virus multiplication occurred. An interferon-like substance was liberated as early as 12 hours after inoculation of this virus. Titers of interferon ranged between 1:256 and 1:1024 as assayed in HA cells challenged with Sindbis or poliovirus. This and other interferons were far less effective in protecting HA cells against the non-transmissible cytopathic effect of Sendai virus. In contrast, however, these cells when previously infected with mumps, SV_5, and DA viruses proved completely resistant to challenge with high concentrations of Sendai virus.

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