Induction of Interferon in Human Lymphoblastoid Cells by Sendai and Measles Viruses

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SUMMARY

Sendai and measles viruses were tested for their interferon-inducing capacity in human lymphoblastoid cells. Sendai virus reproducibly induced considerable amounts of interferon (1 research reference interferon unit/10⁶ cells), but no increase in infectious virus titre was observed. Two Edmonston-Enders strains of measles virus grew very well. The attenuated (A) strain was a good interferon inducer (4 units/10⁶ cells), while the virulent (V) strain induced only minimal amounts at a high multiplicity of infection. Pre-treatment of the cells with 5-iododeoxyuridine (IdUrd) had no effect on the growth of Sendai or EE measles virus and on interferon yields induced by Sendai virus. It slightly potentiated interferon induction by EE measles virus. Partial u.v.-light-inactivation of Sendai virus infectivity resulted in a parallel loss in interferon-inducing capacity.

Current systems for the mass production of human interferon are diploid fibroblasts superinduced with polyriboinosinic acid-polyribocytidilic acid complex [poly (rI)·poly (rC); Havell & Vilcek, 1972; Billiau, Joniau & De Somer, 1972, 1973] and primary human leukocytes induced by Sendai virus (Strander & Cantell, 1966). Both have severe limitations: diploid cells are tedious and expensive to grow, and leukocytes are available in limited amounts. In contrast, lymphoblastoid cells can be propagated in large suspension culture vessels (Moore, 1968) but all those so far examined produced only small amounts of interferon either spontaneously (Haase et al. 1970; Adams et al. 1975) or after induction (Zajac, Henle & Henle, 1969; Strander, Mogensen & Cantell, 1975). A maximal yield of 1000 research reference units/ml has been reported, corresponding to 1 unit/10⁶ cells, whereas 10¹⁻³ units/10⁶ cells have been obtained from leukocytes or fibroblasts. In the present work the induction of interferon in a line of human lymphoblastoid cells by Sendai and measles viruses was studied.

The Namalva CL8-cell line was obtained from Dr H. Strander (Karolinska Sjukhuset, Stockholm, Sweden). Cells were cultured in suspension in a CO₂-incubator, using RPMI 1640 medium, supplemented with 10% heat-inactivated foetal bovine serum (FBS). They were re-fed and split every 48 to 72 h. The human HEp-2 tumour cell line CCL23 (American Type Culture Collection, Rockville, Maryland) and Vero cells (Flow Laboratories, Irvine, Scotland), used for interferon assay and virus infectivity assay respectively, were cultivated in monolayer culture in Eagle’s minimal essential medium (EMEM) supplemented with 10% FBS. Media and sera as well as Dulbecco’s phosphate buffer (PBS) were purchased from Flow Laboratories (Irvine, Scotland).

Sendai virus was propagated in 10-day-old embryonated chicken eggs. After centrifugation of the allantoic fluid at 30000 rev/min for 45 min, the virus pellet was re-suspended in 1/30th of the original volume in PBS. The two strains of measles virus used in our study will be referred to as V (virulent) and A (attenuated). The V-strain was obtained through Dr J. De Smyter and Dr E. De Clercq (Rega Institute, Leuven) from Dr J. Melnick (Baylor...
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University, Houston, Texas), where it was considered as a non-attenuated Edmonston–Enders strain. The A-strain was a commercially available vaccine (Attenuvax) kindly provided by the manufacturer (Merck, Sharp & Dohme, Haarlem, The Netherlands). Sendai virus was titrated either on CCL23 cells in flat bottom Microtitre plates (Falcon, Oxnard, California), or by inoculation of 10-day embryonated chicken eggs. The titre in TCID$_{50}$/ml obtained in CCL23 cells was 1/10 of the titre in EID$_{50}$/ml. Either assay was used, as convenient. Measles virus was assayed in Vero cells in Microtitre plates. Prior to determination of virus yields, the samples were frozen and thawed once. Serial threefold dilutions (100 µl) of virus samples were made in duplicate in Microtitre plates using EMEM as a diluent, and cells (100 µl) were added immediately at 10$^4$–8 cells/well. Plates were incubated at 37 °C for 7 days. The cytopathic effect was read microscopically and confirmed by staining with a 0.5% (w/v) solution of crystal violet in a mixture of formalin/ethanol/0.25% saline (1/10/20, v/v). Results were calculated as TCID$_{50}$/ml.

To study the induction of interferon, Namalva cells were grown, washed, and resuspended to approx. 1 x 10$^6$ cells/ml in medium. Samples of 10 ml were dispensed in glass culture vessels (80 mm high, 24 mm diam.) with a flat bottom and screw cap. Virus suspension (0.2 to 1.0 ml) was added, mixed and left to adsorb for 90 min (Sendai virus) or 4 h (measles virus) at 37 °C. The cells were centrifuged in the culture vessel, washed three times, resuspended in 10 ml of fresh medium and re-incubated. At intervals of 2 or 3 days, the cells were shaken up and samples were taken for cell counting (0.1 ml) and virus titrations (0.5 ml). The cells were then left to sediment; as much as possible of the supernatant medium was harvested, clarified, frozen at −20 °C and used for interferon titration. The cells were re-fed with fresh medium.

Interferon assays were carried out in CCL23 cells. Serial threefold dilutions (100 µl/well) were made in duplicate in flat-bottom Microtitre plates using EMEM as a diluent. On each plate a laboratory interferon standard consisting of human fibroblast interferon super-induced with poly (rI).poly (rC) [Billiau et al. 1973] was included. To each well 100 µl of cell suspension (10$^5$ cells/ml) was added. After an 8 h incubation period, cultures were challenged with 50 µl of vesicular stomatitis virus, New Jersey strain (10$^7.7$ p.f.u./ml as measured on L 929 cells). When the cytopathic effect was complete in virus controls, after incubation for 48 h, the cells were washed with PBS and stained with crystal violet. All results are expressed as research reference units/ml in terms of the M.R.C. reference preparation 69/19.

Fig. 1 shows viable cell counts as determined by trypan blue exclusion (upper panels), extracellular virus yields (middle panels) and interferon production (lower panels) in Namalva cell cultures inoculated under various conditions with Sendai and measles viruses. Part (a) shows results obtained with Sendai virus at a high multiplicity of infection (m.o.i.), namely 10$^2$ EID$_{50}$/cell (10$^{-2}$ TCID$_{50}$/cell). The extracellular virus titre decreased from an initial 10$^{3.5}$ TCID$_{50}$/ml (i.e. residual virus from the inoculum) to undetectable levels. Despite absence of measurable virus multiplication, the number of viable cells gradually declined from the second day till the end of the experiment. Interferon production reached its maximum rate of 1000 units/ml per 48 h in the first 2-day period, but decreased from day 4 post-infection. Both strains of measles virus multiplied in Namalva cells. At a comparable m.o.i. of approx. 10$^{-4}$ TCID$_{50}$/cell (Fig. 1, parts c and d), the V-strain replicated faster and attained a 100-fold higher final titre than the A-strain. Despite this, the V-strain failed to induce measurable amounts of interferon, and even at a higher m.o.i. (10$^{-2}$ TCID$_{50}$/cell) only small amounts were formed (total production in 6 days: 300 units/ml; data not shown). The A-strain, on the contrary, was a good inducer. At a m.o.i. as low as 10$^{-4.2}$ TCID$_{50}$/cell
and after a 4- to 5-day incubation period, the cultures produced interferon at a rate of 1000 units/ml per 48 h. With a higher m.o.i. (10^{-2} TCID_{50}/cell) the cytopathic effect was more marked (Fig. 1, part f) and larger amounts of virus and interferon were produced (final production rate of 3000 units/ml per 48 h). On days 10 and 11, production ceased because nearly all the cells were dead. The total yield over 9 days was 80000 units per culture (10^8 cells), equivalent to a yield of 4 units per 10^9 cells.

Iododeoxyuridine (IdUrd) has been shown to stimulate virus production in certain cells (Green & Baron, 1976). Therefore groups of Namalva lymphoblast cultures were
pre-treated with IdUrd to test whether this would lead to enhanced production of Sendai and measles virus, and hence of interferon. Cultures were exposed to IdUrd (30 μg/ml) for 48 h, and then the cells were centrifuged, counted, re-suspended to $1 \times 10^6$ cells/ml, and inoculated with 1 ml of virus suspension and handled as described above. The results are shown in Fig. 1 (parts b and e). During incubation with IdUrd as well as after its removal the number of viable cells decreased. When the cells were infected with Sendai virus, production of virus or interferon was not increased. When IdUrd-pre-treated lymphoblasts were infected with the A-strain of measles virus, they produced as much virus as infected control cells, but slightly more interferon.

Since u.v.-irradiation of Newcastle disease virus increased its interferon-inducing capacity in chick embryo fibroblasts (Meager & Burke, 1972), we looked for a similar effect in the Sendai virus–Namalva cell system. Five hundred μl of a concentrated Sendai virus suspension ($10^{5.2}$ TCID$_{50}$/ml) were exposed in a Petri dish (diam. 17.5 mm) to an 8 W General Electric u.v. lamp at a distance of 140 mm, equivalent to 81 erg/mm$^2$/s. Cultures were infected with Sendai virus at a m.o.i. of $10^{-2}$ TCID$_{50}$/cell and with the same amounts of virus after irradiation with u.v. for various time intervals. Interferon production was measured between days 2 and 4 post-inoculation, i.e. the time when the production rate was greatest (compare Fig. 1, part a). As shown in Fig. 2, there was a parallel decrease of infectivity and interferon-inducing ability with increase in u.v.-irradiation.

In summary, Sendai virus failed to replicate in Namalva lymphoblasts to any measurable extent. Yet it caused gradual cell destruction and induced interferon over a period of 9 days. Slow destruction of the cells suggests abortive replication of the virus, rather than a direct toxic effect of the input virus. Measles virus replicated to a high titre. The virulent V-strain induced only barely measurable levels of interferon. The attenuated A-strain, on the contrary, induced high levels of interferon. A similar although less accentuated divergence in the interferon-inducing potential of measles virus was reported by De Maeyer & Enders (1965): a chicken embryo cell-adapted Edmonston strain induced twice as much interferon as a human amnion cell-adapted strain, though it grew less well in human amnion cells. The continued production of infectious measles virus in the presence of high concentrations of interferon is concordant with the finding (Adams et al. 1975) that some lymphoblast cell lines seem to be insensitive to their own interferon.
Lymphoblasts, despite their malignant origin and properties, can be considered as a potentially useful source for the mass production of human interferon. The yields (4 units/10^6 cells) we obtained are, to the best of our knowledge, the highest described so far. Yet they are still tenfold lower than those which can regularly be obtained by superinduction of diploid human fibroblasts. This superinduction has given low to moderately high results with lymphoblasts (Tovey et al. personal communication). Our attempts to increase the yields in virus-induced cultures have so far been unsuccessful. In some systems u.v.-irradiation of an inducer virus has enhanced its interferon-inducing potential (Burke & Isaacs, 1958). This procedure was attempted using Sendai virus: the interferon-inducing potential was found to decrease in parallel with the infectivity of the inoculum. Pre-treatment of cells with IdUrd has been reported to enhance the replication of RNA and DNA viruses (Green & Baron, 1976). Application of this procedure to Namalva cells inoculated with Sendai virus did not result in increased virus replication or interferon production. With measles virus (A-strain) the interferon yields obtained in IdUrd pre-treated cells were slightly higher than in untreated cells, although there was no increase in virus production.

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