A Semi-continuous System for the Production of Human Interferon in Lymphoblastoid Cell Cultures

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SUMMARY

The simultaneous occurrence of virus replication and of interferon production in Namalva lymphoblastoid cell cultures infected with measles virus has allowed the development of a semi-continuous production system yielding about $10^4$ units of interferon for each $10^6$ cells.

Human lymphoblastoid cells can be stimulated to produce interferon by synthetic double-stranded RNA (M. G. Tovey et al., personal communication) or by infection with Newcastle disease (NDV) or Sendai virus (Strander et al. 1975; Tovey et al. 1977; Bridgen et al. 1977). Recently measles virus has also been shown to induce interferon in a lymphoblastoid cell line (Volckaert-Vervliet & Billiau, 1977). In contrast to NDV and Sendai virus, which produce abortive infections, measles virus replicates to high titres in lymphoblastoid cells (Gallagher & Flanagan, 1976; Volckaert-Vervliet & Billiau, 1977). The simultaneous occurrence of virus replication and interferon production in such cells opens the possibility for the development of a simple system for producing human lymphoblastoid interferon.

The cells, viruses and methodology used in the present study have been described (Volckaert-Vervliet & Billiau, 1977). Haemagglutination reactions for measles virus were done as described by Shirodaria et al. (1976). All interferon titres are given in terms of the reference standard human interferon preparation, 69/19.

In a first experiment, suspension cultures of Namalva cells (200 ml in 2 l roller bottles at $10^6$ cells/ml in medium RPMI 1640 with 10% foetal bovine serum) were inoculated with the EE (Edmonston-Enders) vaccine strain of measles virus at a multiplicity of infection of 0.001 TCID$_{50}$ per cell. The cultures were incubated on a roller apparatus (30 rotations per hour) at 36.5 °C. Each day the cells were counted using trypan blue staining. When the cell viability had fallen below 50%, the cells were allowed to sediment at 1 g for 2 h at room temperature. The supernatant was harvested and titrated for its interferon and virus content. The sedimented cells were left in the original bottles and 200 ml portions of a fresh suspension of uninfected Namalva cells were added. The bottles were re-incubated and the same procedure was repeated as soon as the cell viability count had again dropped below 50%. From the harvest times shown in Fig. 1, it will be seen that the average duration of a cycle was 3 days. The first harvests contained only small amounts of interferon. However, as the process went on, the titres increased to reach a plateau value of around $10^4$ units/ml. From this time on, 10 units of interferon could be harvested for each $10^6$ cells fed into the system. The increased production of interferon after 15 days in this experiment was not correlated with an increased production of infectious measles virus: fluids harvested at 3 and 18 days contained equal numbers of infectious particles. However, the haemagglutination titre of the later harvest was four-fold higher than that of earlier harvest. This suggests the production during later passages of defective interfering particles such as those described for measles virus in other cells (Rima et al. 1977).

Fig. 2 shows an alternative schedule which gave similar high yields of interferon. In this
Fig. 1. Production of interferon by Namalva lymphoblastoid cells (10⁶ cells/ml) infected with measles virus. At the times shown, supernatant fluid was harvested and assayed for interferon, and fresh cells were added. •, Interferon yield; • infectious virus; □, haemagglutinating activity.

Large-capacity tanks are used in several laboratories at present to culture lymphoblastoid cells, which are subsequently induced to form interferon with Sendai virus or NDV (Bridgen et al. 1977; N. B. Finter et al. personal communication). These inducer viruses are produced separately in embryonated chicken eggs. By using measles virus, the need for such a separate production line can be obviated. In fact, by combining two chemostats, one growing the cells and the second one containing measles virus-infected cells, it should be possible to develop a continuous, fully automated interferon production system. An important factor in the evaluation of such a system is the yield of interferon. Reported optimal yields from Namalva cells range from 2 to 10 units per 10⁶ cells after infection with Sendai virus or NDV (Bridgen et al. 1977; Tovey et al. 1977; N. B. Finter et al. personal communication). Thus, with equal or superior yields (10 units per 10⁶ cells), a continuous measles virus/Namalva cell system has the distinct advantage of obviating the need for separate production of inducer virus.

The profuse replication of measles virus in Namalva cells in the presence of high con-
Fig. 2. Production of interferon by Namalva lymphoblastoid cells (10⁶ cells/ml) infected with measles virus by addition of measles virus infected cells (see text). At the times shown, one-sixth of the culture volume was harvested and replaced by an equal volume of fresh cells. (a) Medium RPMI 1640 with 10% foetal bovine serum; (b) medium alone; (c) medium with 3% of a human plasma protein preparation. Interferon yields.

Concentrations of endogenously generated interferon suggests that in this virus-cell system interferon fails to exert its antiviral action. Several reports have documented the sensitivity of various lymphoblastoid cell lines to the antacellular effect of interferon (Hilfenhaus et al. 1976; Einhorn & Strander, 1977) but there is little or no information about their sensitivity to its antiviral effects. A possible explanation for this lack of information is the fact that most viruses, such as vesicular stomatitis virus, which are used for interferon titration, fail to replicate in lymphoblastoid cells. Other viruses, such as measles virus, replicate relatively slowly and induce the production of interferon, so that the results are difficult to interpret. However, using a virulent measles virus strain which replicates rapidly and fails to induce any measurable interferon, we were able to show that Namalva cells are completely insensitive to the antiviral effect of interferon (G. Volekaert-Vervliet et al. unpublished data). This would explain why a virus that quickly induces interferon can nevertheless continue to replicate over a period of several days.

In this context it can be anticipated that the semi-continuous system that we propose may be applicable to any interferon-resistant lymphoblastoid cell line infected with an effectively replicating virus. Semliki Forest virus is one of the few viruses which has been shown to replicate to high titres in lymphoblastoid cells (Hilfenhaus, 1976) but as yet there is no information about its ability to induce interferon in these cells.
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REFERENCES


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