Interferon Production by a Human Lymphoblastoid Cell Line (DG-75) Free of the Epstein-Barr Virus Genome

ARYE LAZAR,† SHAUL REUVENY, MOSHE MINAI, ABRAHAM TRAUB, AND AVSHALOM MIZRAHI

Biotechnology Department, Israel Institute for Biological Research, Ness Ziona 70450, Israel

Received 18 February 1981/ Accepted 21 May 1981

A new lymphoblastoid cell line, DG-75, was investigated for its ability to produce interferon. DG-75 cells, previously shown to be free of Epstein-Barr virus genome and receptors, could be grown in submerged culture and could produce interferon in titers comparable to interferon produced by Namalva cells. The interferon produced was similar in size to the Namalva interferon as determined by gel filtration in Ultrogel AcA54. The DG-75 cells present a new source for large quantities of interferon which may be safer for human use than the Namalva interferon.

The recent growing use of interferons (IFNs) in clinical trials as antiviral and antitumor agents (5, 16, 18) has resulted in an increased demand for human IFNs. Current methods for the production of IFNs by either viral induction of human peripheral blood leukocytes (3) or superinduction of monolayer cultures of human diploid cell strains (7) are difficult and expensive and do not provide the quantities of IFNs needed for extensive therapeutic trials in humans. The successful transfer of human IFN genes into Escherichia coli (14) constitutes a new source for mass production of human IFN. However, the methodology for obtaining large amounts of highly purified, high-titered bacterial IFN is still to be developed. Lymphoblastoid cell lines constitute another source for large quantities of IFN (19). These cells can be grown in submerged cultures which can be easily scaled-up and provide large quantities of human IFN. The Namalva lymphoblastoid cell line, established by Klein et al. (11), is widely used as a cell substrate for the production of human lymphoblastoid IFN after viral induction. Since the Namalva cells contain parts of the Epstein-Barr virus genome, they are not presently accepted in many countries as IFN producers for clinical trials in humans.

In this paper, a new potential source for human lymphoblastoid IFN assumed to be more suitable for clinical use is reported. A human lymphoblastoid cell line, designated DG-75, lacking the Epstein-Barr virus genome and receptors, was found to be a good IFN producer. The DG-75 cell line was established by Ben-Bassat et al. (1) from the pleural effusion of a child with a primary abdominal lymphoma which resembled Burkitt’s lymphoma. IFN production by these cells was compared with that by the Namalva cells.

MATERIALS AND METHODS

Cells. DG-75 cells were kindly provided by N. Goldblum, Hebrew University, Hadassah Medical School, Jerusalem, Israel. Cells were grown in spinner flasks in RPMI 1640 medium (13) supplemented with 10% fetal bovine serum. Namalva cells were obtained from M. Revel, Weizmann Institute of Science, Rehovot, Israel. The Namalva cells were grown in spinner flasks and in pilot-plant-scale fermentors in an improved RPMI 1640 medium developed in our laboratory (17). Two cell substrates were used for IFN assay: a human foreskin fibroblast cell strain (designated BG-9), established at Roswell Park Memorial Institute, Buffalo, N.Y. (8); and a bovine kidney cell line, Madin-Derby bovine kidney (ATCC CCL-22). Cells were grown as monolayers in Eagle minimal essential medium supplemented with 10% fetal bovine serum.

Viruses. A strain of Sendai virus was kindly provided by K. Cantell of the Central Public Health Laboratory, Helsinki, Finland. The virus was grown in the allantoic cavity of 10-day-embryonated eggs. The harvested virus was kept at –70°C in the presence of 4% gamma globulin-free human plasma. Newcastle disease virus strain B-1 was grown as described above for Sendai virus. Vesicular stomatitis virus was grown in a monolayer culture of L-929 mouse cells. Infectivity and hemagglutination assays were carried out by conventional techniques. The Sendai virus and the Newcastle disease virus strains were used for viral induction of IFN, whereas the vesicular stomatitis virus strain was used as a challenge virus in the IFN assay.

IFN induction. The method used for IFN induction was as follows. Harvested cells, DG-75 or Namalva, were spun down and resuspended in serum-free RPMI 1640 medium at a concentration of 5 × 10⁶ to 10 × 10⁶ cells per ml. The cells were primed with lymphoblastoid IFN at a concentration of 100 U/ml for 2 h. Then Sendai or Newcastle disease virus was added at a concentration of 100 hemagglutination units.
units per ml. The induced cells were incubated at 37°C for 24 h and then removed by centrifugation. The supernatant's pH was adjusted to 2.0 to inactivate the residual virus. After incubation at 4°C for 24 h, the pH was raised to 7.0, and the IFN was stored at −70°C until assay or further processing.

**Gel filtration of IFN.** The method for gel filtration of Namalva IFN was described in detail by Reuveny et al. (17). Briefly, crude IFN was precipitated by 5% trichloroacetic acid, and the precipitated proteins were resuspended in 0.1 volume of potassium phosphate buffer, pH 8.0. After adjustment of the pH to 8.0 with 5 N KOH, the suspension was centrifuged for 1 h at 105,000 × g. The supernatant was applied to a column of Ultrogel AcA54 in 0.1 M potassium phosphate buffer (pH 8.0) containing 0.04% sodium azide. Fractions were collected and assayed for IFN and protein.

**IFN assay.** Two methods of IFN assay were used in this study: the colorimetric technique developed by Finter (4), using BG-9 cells challenged with vesicular stomatitis virus, and the microassay method of Tilless and Finland (20), using Madin-Darby bovine kidney cells challenged with vesicular stomatitis virus. A standard reference human leukocyte IFN (National Institutes of Health G-023-901-527) was included in each assay, and titers were expressed by international reference units.

**Protein determination.** Protein was determined according to the method of Lowry et al. (12) and by fluorometric assay (2), using bovine serum albumin as a standard.

**RESULTS AND DISCUSSION**

**Kinetics of DG-75 cell growth.** The growth characteristics of DG-75 cells were studied to determine conditions yielding maximum cell production. RPMI 1640 medium was found to be superior to both minimal essential medium and McCoy 5A medium. Ten percent fetal bovine serum was optimal for cell growth. Every 2 to 3 days, the cell culture was diluted to 5 × 10⁶ cells per ml in fresh medium. Under these conditions the saturation density of the culture reached 2 × 10⁷ to 3 × 10⁷ viable cells per ml (over 95% viability). When new cultures were established in fresh medium, a 1- to 2-day lag period in growth was observed. At the log phase, a doubling time of 24 h was obtained. Agitation of the culture was necessary to achieve optimal cell growth. Poor growth was observed when cultures were kept stationary. The growth characteristics of the DG-75 cells were found to be similar to those of the Namalva cells, as previously reported by our group (17) and by Zoon et al. (21).

**IFN production by DG-75 cells.** The kinetics of IFN production by DG-75 cells were studied, with Newcastle disease virus and Sendai virus as inducers. In both cases IFN was detected at 5 h after initiation of induction. By 10 h maximum levels of 30,000 to 50,000 IFN units per ml were obtained. No significant changes in IFN titers were observed when induced cultures were assayed at 24 or 48 h. The kinetics of DG-75 IFN production and the titers achieved were similar to those obtained by Namalva cells in our laboratory and by other groups (9, 21).

The IFN yield was independent of serum concentration. No significant differences in IFN titers were observed in cultures containing serum ranging from 0 to 10%. In this feature too, the DG-75 IFN resemble Namalva IFN (9). The stability of crude DG-75 IFN in a frozen state at −70°C was tested for up to 3 months. No significant changes in IFN titers were observed.

The consistency of IFN production by DG-75 cells was studied over a period of 20 weeks in comparison with IFN produced by Namalva cells. There was a difference of more than 10-fold between the highest and the lowest IFN titers obtained over this period (Fig. 1). DG-75 and Namalva cells did not differ significantly in this respect. In early studies, when low-passaged DG-75 cells were used, IFN production in some cases dropped to zero for no known reason. This phenomenon was not observed when later-pas-

![Fig. 1. IFN production by DG-75 (A) and Namalva (B) cells. Spinner cultures of DG-75 and Namalva cells were induced as described in the text at intervals during a 20-week period.](http://aac.asm.org/)
saged cells were used.

**Gel filtration of DG-75 and Namalva IFNs.** Gel filtration of trichloroacetic acid-concentrated crude IFN obtained from DG-75 and Namalva cells was performed in Ultrogel AcA54 columns (Fig. 2). When the filtration was run under the same conditions, DG-75 and Namalva IFNs showed similar profiles, and IFN was collected at the same fractions. The recovery was 80 to 90%. Crude as well as partially purified DG-75 IFN were assayed on Madin-Darby bovine kidney and human fibroblast cells. IFN titers were comparable on both cells, indicating a full cross-reactivity, classifying this IFN in the α group (6).

The results reported in this paper present a new, human lymphoblastoid cell line, free of Epstein-Barr virus genome and receptors. The DG-75 cells can be grown successfully in submerged cultures and produce high titers of IFN after viral induction. The lack of Epstein-Barr virus genome in the cells makes the DG-75 IFN a new potential source for large quantities of IFN suitable for clinical trials.

Further characterization of the DG-75 cells and the induced IFN are currently under way.

**ACKNOWLEDGMENTS**

We are grateful for the skillful technical assistance of Miriam Gez, Pinchas Parnes, and Shlomo Haboussa of the Israel Institute for Biological Research, Ness Ziona, Israel, and to Barbara Grossmayer of Roswell Park Memorial Institute, Buffalo, N.Y. V. Goldblum is kindly acknowledged for supplying the DG-75 cell line. The fruitful discussions with J. A. O'Malley and E. Sulkowski are also acknowledged with thanks.

This research was partially supported by a grant from the National Council for Research and Development, Israel, and the GSF, Munich, West Germany. The Irving Levin Fund is also acknowledged for partially supporting this work.

Part of this work was carried out by A.L. during his stay in the Department of Viral Oncology, Roswell Park Memorial Institute, Buffalo, N.Y.

**LITERATURE CITED**


