Antiviral Research, 1 (1981) 275–280
Elsevier/North-Holland Biomedical Press

THE HUMAN LYMPHOMA CELL LINE NC-37: AN ALTERNATIVE SOURCE OF HUMAN LYMPHOBLASTOID INTERFERON

GUENTHER R. ADOLF, GERHARD BODO and PETER SWETLY

Ernst Boehringer Institut für Arzneimittelforschung, Dr. Boehringer Gasse 5–11, A-1121 Vienna, Austria

(Received 3 July 1981; accepted 14 August 1981)

NC-37 lymphoma cells treated with *n*-butyrate or 5-bromodeoxyuridine (BrdUrd) and induced with Sendai virus produced crude interferon with titers of up to 2×10^5 units/ml (100 units/10^3 cells) and with specific activities of up to 10^6 units/mg protein. More than 90% of the interferon activity was neutralized by antiserum against human interferon type α (HuIFN-α). Treatment of the cells with BrdUrd, but not with butyrate, resulted in the production of moderate amounts of interferon without virus induction.

butyric acid 5-bromodeoxyuridine interferon lymphoblasts

In an early study on interferon production in transformed human haematopoietic cells, Strander et al. [11] observed a wide variation of the interferon response to Sendai virus in 21 lymphoid cell lines. The Burkitt's lymphoma line Namalwa was identified as the best producer of an interferon, which was later shown to consist mainly of HuIFN-α and minor, variable amounts of HuIFN-β [5]. In spite of further, extensive screening programs, Namalwa has so far remained the cell line of choice for the production on a larger scale of human 'lymphoblastoid' interferon.

Interferon production in Namalwa cells can be markedly enhanced by treatment of the cells with one of several chemicals, e.g. *n*-butyrate or 5-bromodeoxyuridine (BrdUrd), all of them inhibitors or inducers of cell differentiation [2–4, 8, 12]. Although these compounds are active not only in Namalwa cells, but also in many other lymphoid cell lines, the extent of stimulation varies widely from one cell line to another (refs. 4, 12 and our unpublished data). We have therefore examined the effects of several chemicals on interferon production in a number of human haematopoietic cell lines, attempting to identify cells which, although not necessarily superior to Namalwa in an untreated condition, would produce more interferon after treatment with chemicals. We report here that a strain of the cell line NC-37 meets these criteria, and may be suitable as an alternative source of human interferon.

NC-37 was until recently considered to be a lymphoblastoid cell line established from the peripheral blood of a healthy donor [6]. Recent studies have revealed, however, that

NC-37 is most probably a subline of Raji (Burkitt's lymphoma) cells that has arisen from contamination of the original cultures (H. zur Hausen, personal communication). NC-37 cells contain approximately 80 Epstein–Barr virus (EBV) genome equivalents per cell [1]; all cells express the EBV-associated nuclear antigen. A small fraction of the cells (< 1%) spontaneously express the EBV early antigen; this percentage is remarkably increased after treatment with chemicals such as BrdUrd, butyrate or phorbol esters. Viral capsid antigen or mature viral particles have never been observed in untreated or treated cells [7].

NC-37, Raji, and Raji 7-7 cells (obtained from Dr. H. zur Hausen, Freiburg) and Raji 6 HAT cells (from Dr. W. Berthold, Biberach) were maintained in a stationary suspension culture in RPMI 1640 medium containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (50 units/ml). If not mentioned otherwise, experiments were performed in 10 ml cultures. Chemicals were added to cultures of rapidly growing cells ($3-5 \times 10^5$ cells/ml); interferon was induced by Sendai virus (approximately 2×10^6 cells/ml, 2^{10} haemagglutinating units virus/ml in medium containing 1% fetal calf serum). Interferon titers were determined in a plaque-reduction assay using GL-V3 vervet monkey kidney cells and vesicular stomatitis virus; all titers were expressed in terms of the international standard of human leukocyte interferon, 69/19. Neutralization assays were carried out by incubating 8–10 units of an interferon preparation (80–100 units/ml) with a 1 : 100 dilution of specific antisera for 60–90 min at 37°C and titration of the residual interferon activity. All titrations and neutralizations were done in duplicate. The antisera used were provided by the National Institute of Allergy and Infectious Diseases, Bethesda, MD, U.S.A., and by Dr. C. Tan, University of Calgary, Canada.

In stationary suspension culture, NC-37 cells grew to maximal densities of about 2.5×10^6 cells/ml with a population doubling time of approximately 24 h. Relatively high interferon titers were already observed when untreated cells were induced with Sendai virus; pretreatment with any of several chemicals active on Namalwa cells resulted in a marked enhancement of interferon yields, with butyrate and BrdUrd being most potent (Table 1). As it is the case with Namalwa cells [2–4], enhancement of interferon synthesis by chemicals was inevitably accompanied by inhibition of DNA synthesis (determined by [^{3}H]thymidine incorporation as described previously [3]) and of cell proliferation (Table 1).

A number of experiments was performed to optimize conditions for interferon production. Concentrations of butyrate higher than 1 mM did not further enhance interferon yields, and 2 mM butyrate sometimes gave even lower titers. In several experiments, interferon production was induced after 26, 48 and 72 h of treatment with 1 mM butyrate or 50 μg/ml BrdUrd; in all cases, optimal enhancement was observed already after 26 h in the presence of the chemicals. Furthermore, combined treatment with butyrate (1 mM) and BrdUrd (50 μg/ml) for 48 h did not result in a stronger enhancement than treatment with butyrate alone (data not shown).

For a preliminary characterization of interferon produced by NC-37 cells pools were prepared from interferon induced in butyrate- or BrdUrd-treated cells (six independent

TABLE 1

Effects of chemicals on DNA synthesis and interferon production in NC-37 cells

Treatment		Thymidine incorporation (% of control) Mean ± S.D. (n = 3)	Interferon yield			
			Units/10^3 cells			Enhancement (mean)
			Exp. 1	Exp. 2	Exp. 3	
Control		100	1.5	1.4	3	–
Butyrate	10^{-3} M	4 ± 3	64	52	59	33
Dexamethasone	10^{-5} M	16 ± 7	7	3.5	15	4
Dimethylsulfoxide	2.8×10^{-2} M	2 ± 0	11	6	11	5
TPA	1.7×10^{-7} M	43 ± 20	10	17	13	7.5
BrdUrd	1.6×10^{-4} M	n.d.[a]	25	55	61	26

Cells were induced for interferon production after 48 h of treatment as described in the text. DNA synthesis was determined by pulse-labelling with tritiated thymidine as described previously [3] (data from three independent experiments).

[a] Not determined.

experiments). Comparative titration of these preparations on monkey (GL-V3) and human (WISH) cells gave a high ratio of activities indicative of a high proportion of α-type interferon. Neutralization assays using antisera specific for HuIFN-α and -β revealed that butyrate- as well as BrdUrd-treated cells produced more than 90% IFN-α (data not shown).

A few experiments were performed as a first approach to scale up interferon production with NC-37 cells. Stationary suspension cultures with volumes of up to 200 ml were grown in plastic flasks, larger cultures were grown in Erlenmeyer flasks on rotatory shakers or in roller bottles. Although the highest yields (up to 10^5 units/10^6 cells) were obtained from stationary cultures, cells grown in roller bottles still produced large amounts of interferon, and up to 1.5×10^7 units could be obtained from a single bottle. Moreover, unfractionated supernatants already contained interferon with very high specific activity (up to 1.2×10^6 units/mg protein). Compared with Namalwa cells, NC-37 clearly gave rise to higher titers as well as higher specific activities in the crude supernatants (Table 2).

As indicated in the introduction, NC-37 cells most probably are derived from the Raji line of Burkitt's lymphoma cells. We have therefore examined the original Raji cell line as well as two independently derived clones (Raji 6 HAT and Raji 7-7) with respect to interferon production and response to chemicals. In our hands, neither of these cell lines produced measurable quantities of interferon in response to Sendai virus. Following pretreatment with butyrate or BrdUrd, moderate amounts of interferon were produced (20–500 units/10^6 cells), whereas dexamethasone, dimethylsulfoxide or 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) were ineffective (data not shown).

Several human lymphoid cell lines (including NC-37) have been described to produce interferon without induction by viruses or nucleic acids, although in most cases at a very low level ('spontaneous' or 'autogenous' interferon) [9, 10]. Interferon activity in spent media of our NC-37 cultures was, however, consistently below our level of detection (i.e. $\leqslant 1-2$ units/ml) and remained so after treatment of the cells with butyrate, dexamethasone or dimethylsulfoxide for up to 5 days; marginal interferon activity was in some experiments detected after treatment with TPA (up to 5 units/ml). In contrast, BrdUrd (50 or 100 μg/ml) in all experiments 'induced' production of substantial amounts of interferon in accordance with earlier reports [9, 12], but maximal production rates (150–350 units/10^6 cells in 24 h) were only observed after 4–7 days of treatment (data not shown). Since the enhancement by BrdUrd of virus-induced interferon production is maximal already after 26 h of treatment, these data indicate that different mechanisms are involved in the stimulatory effects of BrdUrd on spontaneous and virus-induced interferon synthesis. This would also explain why butyrate is at least as potent as BrdUrd with regard to enhancement of virus-induced interferon production, but does not stimulate spontaneous interferon synthesis.

TABLE 2

Interferon production in NC-37 and Namalwa cells treated with butyrate

Type of culture	Cell line	Experiment No.	Cell density in production stage (cells/ml × 10^{-6})	Interferon titer (units/ml × 10^{-3})	Specific activity (units/mg × 10^{-6})
Stationary suspension (200 ml/culture)	NC-37	1	2	200	–[a]
		2	2	40	–
Shaken suspension (400 ml/culture)	NC-37	1	5	44	–
		2	5	26	–
		3	5	82	–
	Namalwa	1	5	23	–
		2	5	9	–
		3	5	18	–
Roller bottles (800 ml/culture)	NC-37	1	2	44	1.22
		2	5	27	0.45
		3a[b]	5	68	0.62
		3b	5	97	0.97
	Namalwa	1	5	10	0.18
		2	5	12	0.17
		3	5	7	0.20

After treatment with 1 mM butyrate for 48 h the cells were incubated with Sendai virus (2^{10} units/ml) for 2 h at cell densities of 2×10^7 or 5×10^7/ml, the centrifuged, suspended in serum-free medium and incubated for another 20 h.

[a] Not determined.

[b] Parallel cultures without priming (3a) and primed with 500 units/ml (3b).

ACKNOWLEDGEMENTS

We thank Drs. H. zur Hausen and W. Berthold for cell lines and Dr. Y. Tan and the National Institutes of Health for antisera. The excellent assistance of Ms. M. Kirchbaumer is gratefully acknowledged.

REFERENCES

1 Adams, A. (1979) The state of the virus genome in transformed cells and its relationship to host cell DNA. In: The Epstein–Barr Virus. Eds.: Epstein, M.A. and Achong, B.G. (Springer Verlag, Berlin–Heidelberg–New York) pp. 156–183.

2 Adolf, G.R. and Swetly, P. (1979) Interferon production by human lymphoblastoid cells is stimulated by inducers of Friend cell differentiation. Virology 99, 158–166.

3 Adolf, G.R. and Swetly, P. (1979) Glucocorticoid hormones inhibit DNA synthesis and enhance interferon production in a human lymphoid cell line. Nature 282, 736–738.

4 Adolf, G.R. and Swetly, P. (1980) Tumor-promoting phorbol esters inhibit DNA synthesis and enhance virus-induced interferon production in a human lymphoma cell line. J. Gen. Virol. 51, 61–67.

5 Dalton, B.J. and Paucker, K. (1979) Antigenic properties of human lymphoblastoid interferons. Infect. Immun. 23, 244–248.

6 Durr, F.E., Monroe, J.H., Schmitter, R., Traul, K.A. and Hirshaut, Y. (1970) Studies on the infectivity and cytopathology of Epstein–Barr virus in human lymphoblastoid cells. Int. J. Cancer 6, 436–449.

7 Fresen, K.O., Cho, H. and Zur Hausen, H. (1978) Recovery of transforming EBV from non-producer cells after superinfection with non-transforming P3HR-1 EBV. Int. J. Cancer 22, 378–383.

8 Johnston, M.D. (1980) Enhanced production of interferon from human lymphoblastoid (Namalwa) cells pretreated with sodium butyrate. J. Gen. Virol. 50, 191–194.

9 Klein, G. and Vilcek, J. (1980) Attempts to induce interferon production by IdUrd induction and EBV superinfection in human lymphoma lines and their hybrids. J. Gen. Virol. 46, 111–117.

10 Pickering, L.A., Kronenberg, L.H. and Stewart II, W.E. (1980) Spontaneous production of human interferon. Proc. Natl. Acad. Sci. U.S.A. 77, 5938–5942.

11 Strander, H., Mogensen, K.E. and Cantell, K. (1975) Production of human lymphoblastoid interferon. J. Clin. Microbiol. 1, 116–117.

12 Tovey, M.G., Begon-Lours, J., Gresser, I. and Morris, A.G. (1977) Marked enhancement of interferon produced in 5-bromodeoxyuridine treated human lymphoblastoid cells. Nature 267, 455–457.