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Production of Human Lymphoblastoid Interferon

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The interferon response of 21 lines of human lymphoblasts varied greatly. Interferon from the best producer (11,000 U/ml) resembled human leukocyte interferon.

Peripheral blood leukocytes have been the source of human interferon (IF) for clinical trials (6, 10). The yields obtained from these cells have increased considerably during the past 10 years (K. Cantell, S. Hirvonen, K. E. Mogensen, and L. Phyälä, In Vitro, in press), but large-scale production will always be limited by the availability of human blood. As a source of potent IF, human lymphoblastoid cells have received scant attention (1). As continuous lines, growing well in suspension, these cells would be suitable for the mass production of human IF. One line, RPMI 1196, was studied in our laboratories (1), but little IF was obtained. This note reports the screening of 21 human lymphoblastoid lines and the comparison of IF from one such line with other human IFs.

The human lymphoblastoid lines were obtained through the courtesy of George Klein, Department of Tumor Biology, Karolinska Institutet, Stockholm 60, Sweden. Kamoti and Odour lines were recently established from Burkitt lymphomas. The other lines are described by Klein et al. (4), and the five hybrids (RN) of Raji and Namalva by Nyormoi et al. (9). The lines were propagated as stationary suspension cultures in RPMI 1640 medium, with 15% fetal calf serum, and fed twice weekly.

For screening, the cells were adjusted to 10^7/ml in growth medium. A 5-ml amount of cell suspension was incubated in a 100-ml conical flask with 600 hemagglutination units of live Sendai virus per ml. After 24 h at 37 C, the supernatant fluid was dialyzed against buffer at pH 2 for 24 h, followed by back dialysis to neutrality before assay (2). All IF units are expressed in terms of the international reference preparation 69/19 (National Institute of Medical Research, London). Nearly one-half of the cell lines produced no detectable IF, and the yields from the others varied considerably (Table 1).

The Namalva line, which gave the best titers, was studied further. These cells have a doubling time of 30 h, and they grow to a maximal density of about 5×10^6 cells/ml. Cells at 10^7/ml were induced by the methods described for blood leukocytes (2; Cantell et al., in press). The experiments were done in 25-ml tubes, 100-ml Kjeldahl flasks, or 2,000-ml round-bottomed flasks. The containers had at least 50% air space and were loosely covered with foil. Cultures were primed by adding 100 U of human leukocyte IF per ml, 1 h before the addition of live Sendai virus. Increasing levels of Sendai virus improved IF production by Namalva cells (Table 2). We expect that better yields can be obtained when systematically screened cell lines are carefully studied to find the optimal conditions for IF production.

Human leukocyte IF differs from the IF produced by cultured human diploid fibroblasts; leukocyte IF is more stable (3) and there are antigenic differences (5). IF from Namalva cells resembles that from leukocytes rather than that from diploid fibroblasts (Table 3). The values obtained for the neutralizing titers (7) of a rabbit anti-IF serum suggest that IFs of lymphoblastoid and leukocytic origin may have similar antigenic determinants.

Namalva and leukocyte IFs are resistant to guanidine (3, 8) and sensitive to mercaptoethanol (8). Activity lost due to mercaptoethanol is recovered by the addition of guanidine (8). Sodium dodecyl sulfate interferes with this recovery (8), although it is itself without adverse effect on these interferons (Cantell et al., in press). Namalva and leukocyte IF can be partially purified by the same scheme of selective precipitation from 94% ethanol (Cantell et al., in press).

It would thus appear that human IF can be prepared in comparatively good yield from certain lymphoblastoid lines, and that such a preparation might combine the desirable characteristics of leukocyte IF with the advantages

of production from a continuous cell line in suspension culture.

TABLE 1. *IF response of 21 human lymphoblastoid cell lines*

Cell line	IF (U/ml)
Namalva	600
Akuba	200
P3HR-1	200
LY-46	200
Kamoti	60
Maku	60
NC37	60
SKL-1	60
DSTC4	20
JHTC33	20
Naliaka	20
Raji	20
Daudi	<20
Odour	<20
PE-SS	<20
6410	<20
RN13	<20
RN16	<20
RN20	<20
RN21	<20
RN HAT	<20

TABLE 2. *Effect of Sendai virus concentration on the production of IF by Namalva cells*

Sendai virus (HA[a] U/ml)	IF (U/ml) produced from:		
	25-ml tubes	100-ml flasks	2,000-ml flasks
30		1,100	
60	3,500		
100[b]		3,500	
300	3,500		11,000
600		6,000	
1,000	11,000		
2,000		11,000	
6,000	11,000		

[a] HA, hemagglutination.

[b] This concentration gives maximal titers (50,000 U/ml) with peripheral blood leukocytes (3).

TABLE 3. *Comparison of Namalva IF with human IF from other sources*

Source of crude IF	Original activity[a] (%) after:		Neutralizing titer (rabbit anti-IF serum)[b]
	1 h	24 h	
Namalva, lymphoblasts	60	20	1:30,000
Peripheral blood leukocytes	60	20	1:30,000
Diploid fibroblasts	30	3	1:550

[a] Conditions: 56 C, pH 4.0.

[b] Pool prepared by immunization of several rabbits with partially purified human leukocyte IF (15 weekly injections of 3×10^6 U, subcutaneously without adjuvant).

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