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Some Properties of Virus and Immune-induced Human Lymphocyte Interferons

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Small lymphocytes respond *in vitro* to virus infections with the synthesis of interferon. But viruses and their nucleic acids are not the only inducer. Recently we have demonstrated that specific antilymphocyte globulins (ALG) (Falcoff *et al.* 1972*a*; Falcoff, Oriol & Iscaki, 1972*b*), and its divalent $F(ab')_2$ fragments (Falcoff *et al.* 1972*b*), are also able to induce interferon synthesis. A number of unrelated non-virus substances share this property: phytohaemagglutinin (Wheelock, 1965), concanavalin (Falcoff, unpublished results), etc.

The most remarkable difference between the two groups of inducers is the nature of the target cell: while viruses are active on many kinds of cultured cells, including lymphocytes, the second group is only active on immunocompetent cells.

The present study was undertaken in an attempt to distinguish the antivirus substance synthesized by ALG-stimulated human lymphocytes from the interferon induced in similar cultures by irradiated Newcastle Disease Virus (NDV).

Methods for culture of human peripheral lymphocytes and the conditions of stimulation by ALG have been given in detail elsewhere (Falcoff *et al.* 1972*a*). NDV (HERTS strain) irradiated at 5000 erg/mm.^{2} with u.v. light with maximum emission at 2537Å was used as virus inducer, at a multiplicity of 50 p.f.u./cell. After 48 hr incubation, cultures were centrifuged, the supernatant fluids dialysed against pH 2 buffer for 5 days, then adjusted to pH 7·2 and ultracentrifuged. Titrations of interferon were carried out on primary human amnion cultures with VSV as challenge virus, as already described (Falcoff *et al.* 1966).

Table 1 summarizes the results of the interferon production by both inducers, on cultured lymphocytes from the same donor. The species-specific antivirus activity was demonstrable in human amnion cells or human diploid WI-38 cells but not in primary embryo cells, hamster cell BHK or mouse L cell cultures. Actinomycin D at a non-toxic level (1 μg./ml.) blocked interferon action.

Table 2 shows some physico-chemical properties of the induced interferons. The stability to enzymatic action and heat were similar for both interferons. However, while the interferon induced by virus was stable at pH 2, interferon induced by ALG was almost completely inactivated at the same pH. The sensitivity to acid seems to be a particular property of interferons not induced by virus (Kleinschmidt & Murphy, 1965; Borecky *et al.* 1967).

Mol. wt, determined by gel chromatography through Sephadex G-100, were also different: 50,000 (± 5000) for the ALG-interferon and 25,000 (± 2000) for the NDV-interferon.

The chomatographic behaviour on DEAE-cellulose suggests that ALG-interferon may be a more basic protein than the virus one. The latter was retained by the column in 0·01 M-phosphate buffer pH 7·8 while the former was excluded. With higher pH (0·01 M-tris, pH 8) both proteins were retained and eluted at increased molarity of NaCl.

At 0·09 M-NaCl the ALG-interferon was eluted while the peak of virus-interferon was found at 0·12 M (Fig. 1). Interferon synthesized by lymphocytes stimulated by PHA showed the same properties as the ALG preparation (Falcoff, unpublished results).

We conclude that similar cultures of lymphocytes are able to produce, depending on the inducer, two distinct proteins with anti-virus activities.

Table 1. *Interferon production by human lymphocyte cultures treated with u.v.-irradiated NDV or H-ALG*

	Inducer	
	u.v. NDV	H-ALG
Titre of interferon	512	1024
Increase in ^{3}[H]-uridine uptake	Not significant	5-fold
1 μg. actinomycin/ml. culture	Interferon not detectable	Interferon not detectable

Table 2. *Physico-chemical properties of two lymphocyte interferons*

	NDV-interferon	ALG-interferon
Mol. wt	25,000	50,000
Treatment	Recovery (%)	
Trypsin	10	5–10
RNase	100	100
DNase	100	100
pH 4	100	12·5
pH 2	100	< 1
56°, 10 min.	100	100

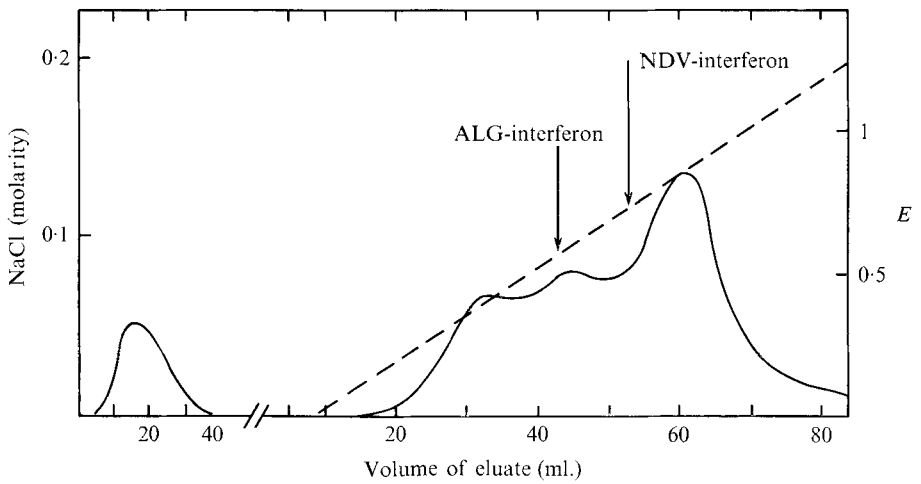

Fig. 1. Five ml. interferon preparations were dialysed for 18 hr against 1 l. of 0·01 M-tris buffer pH 8 and then passed through a DEAE cellulose column (10 × 150 mm.), previously equilibrated with the same buffer. After elution with about 50 ml. of this buffer, the molarity on NaCl of the eluant was gradually increased using a nine-chamber varigrad. ——, E; – –, NaCl molarity; Arrows: interferon peaks.

Interferon has been found to be induced by a variety of non-virus inducers. In additon to those already mentioned, PHA and ALG, the divalent agglutinating fragment $F(ab')_2$ obtained by pepsin cleavage of ALG also acted as an inducer, while monovalent Fab or Fab' did not (Falcoff *et al.* 1972*b*). In other specific immune reactions, like the 'mixed lymphocyte reaction' (Gifford, Tibor & Peavy, 1971) or when sensitized lymphocytes were mixed with the corresponding antigen (Green, Cooperband & Kibrick, 1969), interferon has been detected.

Hence, the synthesis of interferon by immunocompetent cells under sets of circumstances which certainly represent true immune responses, suggests that this interferon can be called immune interferon. The name of virus interferon can then be given to the antivirus protein induced by virus infection of any kind of cell.

The two types of interferon seem to be induced by different mechanisms. For, as we have recently demonstrated, the immune interferon is induced in circumstances in which agglutination takes place, suggesting that the cell-to-cell contact is an essential factor in lymphocyte stimulation; with NDV as inducer, no significant lympho-agglutination can be seen. Another difference is that the virus is supposed to penetrate into the cell, while it has been reported (Greaves & Bauminger, 1972) that immobilised PHA retains its stimulating activity.

The population of circulating lymphocytes is composed of two kinds of cells: the T (thymus derived) cells, implicated in cellular immunity, and the B cells, derived from bone marrow, which play the major role in the synthesis of humoral antibodies. Experimental data on the thymectomized or irradiated animals suggest that the mitotic activity of PHA takes place in T cells and it is proposed that the immune interferon is synthesized by the thymus processed cells. On the other hand, it is not possible to decide which cell is implicated in the synthesis of virus lymphocyte interferon until T and B cells can be separated.

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