

## 2', 5'-Oligoadenylate synthetase induction in lymphocytes of patients with connective tissue diseases

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**SUMMARY** Spontaneous production of the interferon induced enzyme 2', 5'-oligoadenylate synthetase (2', 5'-A synthetase) was significantly greater in blood lymphocytes of patients with systemic lupus erythematosus (SLE), Behçet's syndrome, and rheumatoid arthritis than in control lymphocytes. Alpha interferon (IFN- $\alpha$ ) induced comparable production of enzyme in normal, rheumatoid, and Behçet's lymphocytes, and reduced, but still appreciable amounts in SLE lymphocytes. Phytohaemagglutinin (PHA) did not induce enzyme. Thus spontaneous production of 2', 5'-A synthetase is encountered in several connective tissue diseases and seems likely to be induced by in-vivo exposure to interferons.

**Key words:** rheumatoid arthritis, SLE, Behçet's syndrome, interferon.

The enzyme 2', 5'-oligoadenylate synthetase (2', 5'-A synthetase) is induced by interferons as an essential step in limiting virus growth.<sup>1,2</sup> Raised 2', 5'-A synthetase concentrations have been detected in blood lymphocytes of patients with several virus infections and autoimmune diseases,<sup>3</sup> although it now appears that there is some increase in enzyme concentration in patients with bacterial infections.<sup>4</sup> These observations complement the many reports of interferon-like activity in the serum of patients with SLE,<sup>5</sup> though studies of this kind have given conflicting results.<sup>6</sup> The subject is important for two reasons. Firstly, interferons influence immune responses and have been implicated in the disturbance of immune reactions characteristic of these disorders. Secondly, analysis of the stimuli which induce lymphocyte 2', 5'-A synthetase and circulating interferons is one approach to screening cells or tissues containing putative aetiological agents or their antigens. In this report we show that assaying lymphocyte 2', 5'-A synthetase is a reliable indication of exposure to interferons in several connective tissue diseases.

Accepted for publication 5 August 1985.

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### Patients and methods

Mononuclear cells were separated from heparinised blood by density gradient sedimentation on Ficoll-Hypaque and washed in RPMI 1640, bicarbonate-buffered medium.  $1.0 \times 10^6$  cells/ml ( $1.0 \times 10^9$ /l) of the same medium containing 2% calf serum (Gibco), glutamine, *N*-2-hydroxyethylpiperazine-*N*<sup>1</sup>-2-ethanesulphonic acid (HEPES), penicillin, and streptomycin were cultured in round bottom tubes at 37°C in a 5% CO<sub>2</sub> in air incubator. Experimental results are based on duplicate cultures.

### CELL STIMULATION

Cultures were exposed to alpha interferon (IFN- $\alpha$ ) obtained as partially purified human lymphoblastoid interferon (Wellcome Laboratories, Beckenham, Kent) or 1  $\mu$ g PHA (Burroughs Wellcome). After culture the cells were harvested by centrifugation, washed twice with ice-cold phosphate-buffered saline, and the pellets stored at -70°C.

### 2', 5'-A SYNTHETASE DETERMINATION

Cell pellets were lysed for 10 min on ice in 20  $\mu$ l buffer containing 50 mM KCl, 1.5 mM magnesium acetate, 10 mM dithiothreitol, 10% v/v glycerol, 0.1% nonidet P40, and 10 mM HEPES pH 7.5

(buffer A). The contents were mixed on a rotary mixer and then centrifuged for two min in an Eppendorf centrifuge. 20  $\mu$ l aliquots of cell lysates were distributed into Eppendorf tubes containing 20  $\mu$ l of agarose beads coupled to poly I, poly C (PL Biochemicals).

After mixing, the contents were incubated for 1 h at room temperature. The beads were washed six times in buffer A and incubated for a further 30 minutes before a final wash. 20  $\mu$ l buffer A containing 3 mM adenosine triphosphate (ATP) was added to each tube and incubated for 17 h at 30°C. The product 2', 5'-oligoadenylate was assayed by a radiobinding assay.

#### RADIOBINDING ASSAY

The probe, 2', 5'-A tetramer 5-triphosphate-3' ( $^{32}$ P)pCp (phosphate cytidine phosphate) (Amersham International), specific activity 3000 Ci/mmol was diluted with ATP buffer (5 mM Mg acetate, 20 mM trometamol (TRIS) pH 7.6, 5% v/v glycerol in 1 mM ATP) to give 400 cpm/ $\mu$ l. 8  $\mu$ l probe, 2  $\mu$ l sample, and 10  $\mu$ l S10 (cell lysate from Ehrlich Sarcoma cells diluted in buffer A to give optical density 50 at 280 nm) were incubated on ice in microEppendorf tubes for 90 min. The samples were assayed undiluted and at dilutions of 1/10 and 1/100. The mixtures were spotted onto nitrocellulose filters (Sartorius 0.45  $\mu$ m pore size), washed in distilled water, dried, and counted in econofluor. Standards

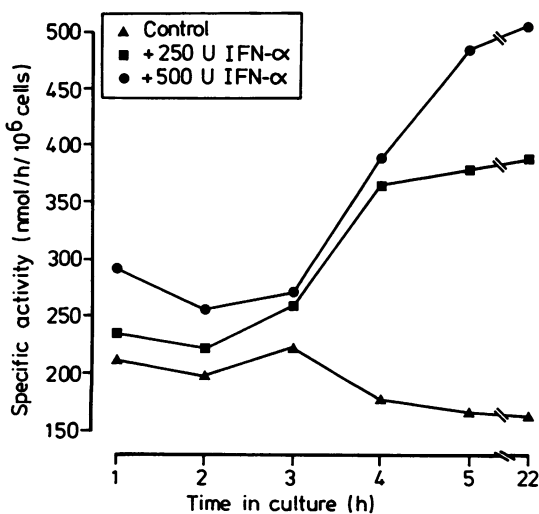


Fig. 1 2', 5'-A synthetase induction by IFN- $\alpha$  in normal lymphocytes. Normal blood lymphocytes were cultured in isolation (▲) or after exposure to 250 U (■) or 500 U (●) IFN- $\alpha$ . Points are means of triplicate assays using cells from a single donor.

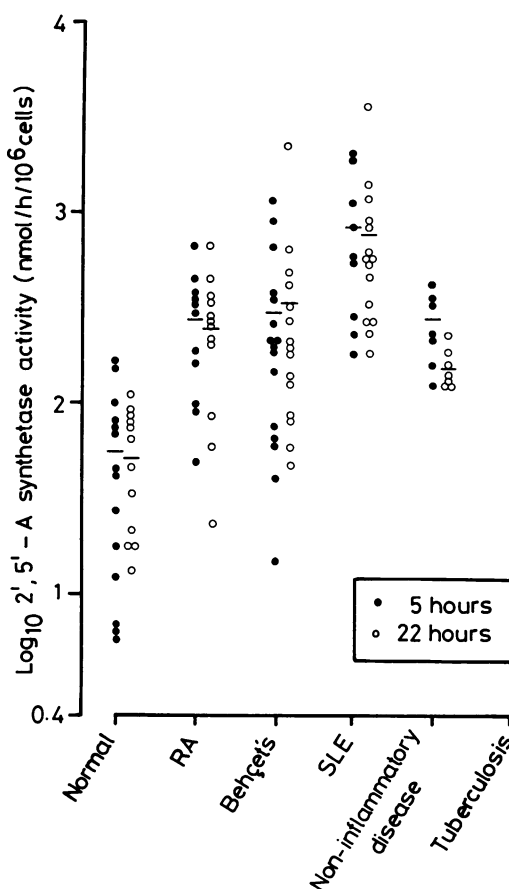


Fig. 2 Basal 2', 5'-A synthetase concentrations in connective tissue disease lymphocytes: (●) five hour concentrations; (○) 22 hour concentrations; horizontal bars indicate 'mean' value.

of varying concentrations of 2', 5'-A synthetase were assayed in all experiments. Enzyme concentration was expressed as enzyme activity (nmol/h/10<sup>6</sup> cells).

#### PATIENT GROUPS

Fourteen patients (nine female, five male) with SLE satisfying the revised American Rheumatism Association criteria<sup>7</sup> were studied (age range 14–69 years), of whom six were receiving prednisolone. Disease activity was assessed on a published numerical scale.<sup>8</sup>

Thirteen patients (seven female, six male) with classical rheumatoid arthritis were studied (age range 22–64 years), of whom two were receiving penicillamine and the remainder non-steroidal anti-

Table 1 Effect of IFN- $\alpha$  on lymphocyte 2', 5'-A synthetase\*

Disease category	No in group	5 hours			22 hours		
		Resting	IFN- $\alpha$	Increase†	Resting	IFN- $\alpha$	Increase†
Rheumatoid arthritis	13	2.323	2.697	0.374	2.363	3.169	0.806
Behçet's syndrome	17	2.328	2.653	0.325	2.296	3.087	0.791
SLE	14	2.777	3.160	0.383	2.691	3.078	0.387
Disease controls	10	2.483	2.889	0.406	2.255	2.910	0.655
Normal controls	14	1.496	2.121	0.625	1.640	2.313	0.673

\*Values are  $\log_{10}$  enzyme activity (nmol/h/ $10^6$  lymphocytes).

†The standard error of the increase is  $\leq 0.128$ .

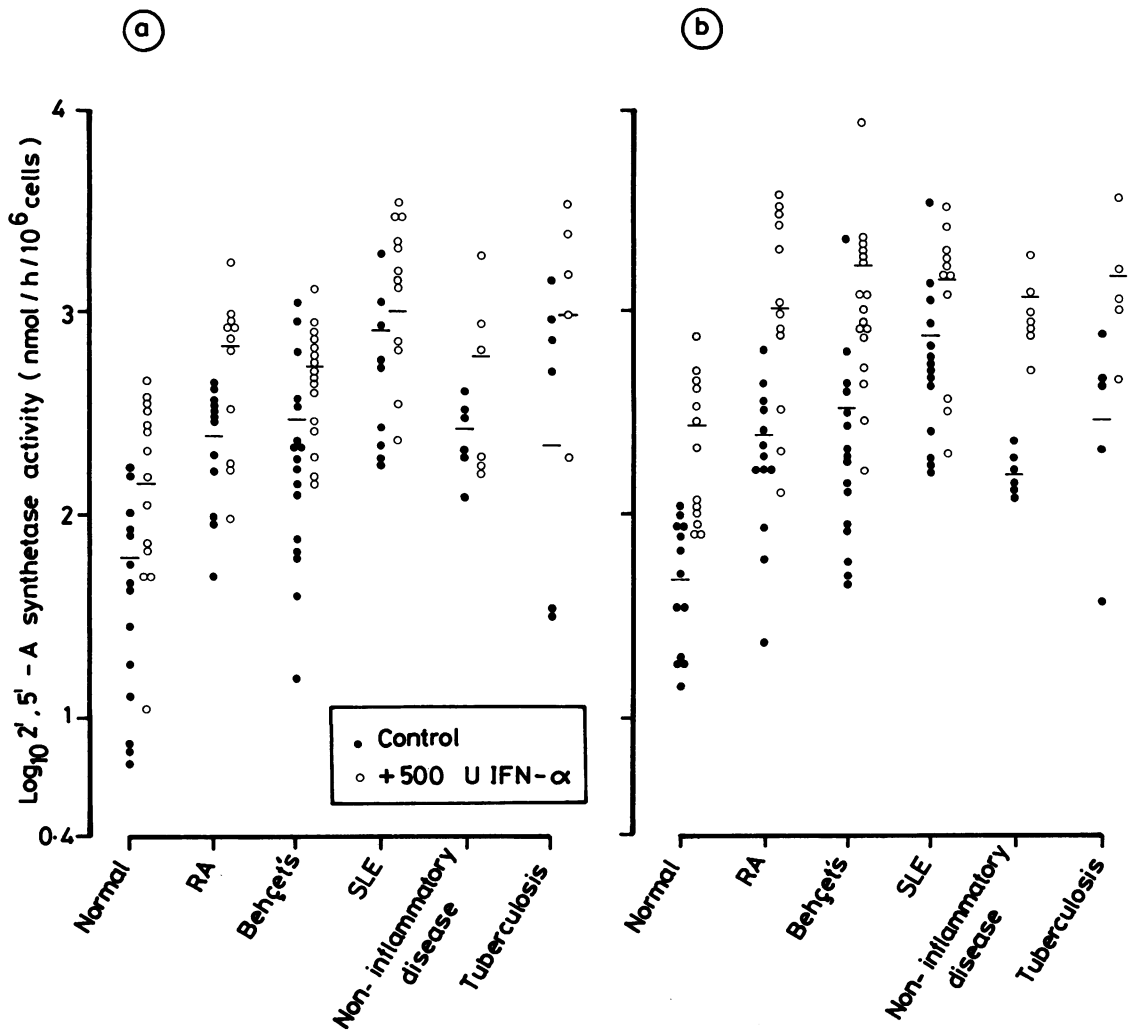


Fig. 3 2', 5'-A synthetase induction by IFN- $\alpha$  in connective tissue disease lymphocytes: (a) five hour concentrations; (b) 22 hour concentrations; (●) unstimulated; (○) + 500 U IFN- $\alpha$ .

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Table 2 Effect of PHA on lymphocyte 2', 5'-A synthetase\*

Disease category	No in group	5 hours			22 hours		
		Resting	PHA	Increase†	Resting	PHA	Increase†
Rheumatoid arthritis	11	2.328	2.285	-0.043	2.378	2.474	0.096
Behçet's syndrome	16	2.322	2.220	-0.102	2.269	2.525	0.256
SLE	13	2.777	2.657	-0.120	2.691	2.723	0.032
Disease controls	8	2.426	2.435	0.009	2.226	2.527	0.301
Normal controls	11	1.493	1.564	0.071	1.626	1.677	0.051

\*Values are log<sub>10</sub> enzyme activity (nmol/h/10<sup>6</sup> lymphocytes).

†The standard error of the increase is ≤0.111.

inflammatory drugs. Disease activity was judged by joint scores and erythrocyte sedimentation rate.

Seventeen patients (10 female, seven male) with Behçet's syndrome were included (age range 36–66 years), all of whom had recurrent orogenital ulcers and one or more of the systemic features included in the diagnostic criteria of Mason and Barnes.<sup>9</sup> Three patients were taking 5 mg prednisolone daily and the rest were off treatment. Disease activity was graded by the criteria of Denman *et al.*<sup>10</sup>

Controls with other diseases comprised three untreated patients (all female) with urticaria (age range 30–50 years), five patients (all female) with polymyalgia rheumatica (age range 50–64 years), and five patients (one female, four male) with pulmonary tuberculosis (age range 23–73 years), all of whom were taking antituberculous drugs.

Fourteen members of the institute staff (nine females, five males, age range 27–50 years) served as controls. This project was approved by the Institute's Ethical Committee.

#### STATISTICAL METHODS

The analysis of variance of the log transformed specific activity was performed for interferon and PHA stimulation separately with the following factors: patient group, duration of culture (five or 22 hours), and stimulation (present or absent). Group comparisons were assessed relative to the variation between patients, while the effects of duration of culture, stimulation, and their interactions with patient groups were assessed relative to the within patient variability.

#### Results

##### SPONTANEOUS PRODUCTION OF 2', 5'-A SYNTHETASE

Spontaneous enzyme activity was maximal in the first few hours of culture and thereafter decreased (Fig. 1). Accordingly the results were analysed after

five and 22 hours of culture. Basal concentrations of 2', 5'-A synthetase in normal lymphocytes were low (Fig. 2). In contrast, high levels of spontaneous activity were found in SLE patients. Increased enzyme concentrations were also present in lymphocytes from patients with Behçet's syndrome and rheumatoid arthritis. However, these were less pronounced and similar increases were observed in lymphocytes from control patients in other diseases (Table 1). There was no correlation between enzyme concentration and disease features or activity in any of these patient groups. Nor did drug treatment influence the results.

##### INDUCTION OF LYMPHOCYTE 2', 5'-A SYNTHETASE BY IFN- $\alpha$

The amount of 2', 5'-A synthetase induced by IFN- $\alpha$  was dose dependent and time dependent (Fig. 1). 500 U IFN- $\alpha$  was used as the standard inducing dose, and enzyme activity was measured five and 22 hours after stimulation.

IFN- $\alpha$  induced enzyme activity in lymphocytes of all patient groups which was detectable after five hours (Fig. 3a) and 22 hours (Fig. 3b) of exposure. Although substantial, this increase was significantly lower in lupus lymphocytes than in cells from the other disease groups or controls.

##### EFFECTS OF PHA STIMULATION ON 2', 5'-A SYNTHETASE CONCENTRATION

PHA induced a small increase in enzyme concentration in lymphocytes from patients with rheumatoid arthritis and Behçet's syndrome (Table 2), but this was slight compared with the response to IFN- $\alpha$ . The increase observed in other groups was small and not statistically significant.

#### Discussion

These observations confirm that increased concentrations of 2', 5'-A synthetase are found in

circulating blood lymphocytes of patients with SLE and rheumatoid arthritis and also show that similar changes are present in lymphocytes from patients with Behçet's syndrome. However, contrary to earlier claims that such increases are confined to patients with autoimmune or viral infections,<sup>3</sup> increased concentrations were also found in lymphocytes from patients with active tuberculosis, in agreement with the recent observations of other observers in bacterial infections.<sup>4,11</sup> The highest concentrations were found in patients with SLE, possibly reflecting greater exposure of blood lymphocytes to circulating interferons.<sup>5</sup> No obvious correlation could be observed between disease features or disease activity. However, these might emerge in studies of larger numbers of patients.

Given the wide range of disorders associated with increased enzyme concentrations in blood lymphocytes, these observations do not help to define possible aetiological agents in connective tissue disease. Furthermore, since there are numerous pathways by which immune responses induce interferon production, enzyme induction could simply reflect the characteristic immunoproliferative events in these diseases, irrespective of their cause. Nevertheless, our results show that enzyme induction in diseases such as SLE is more likely to reflect prolonged exposure to interferons than other mechanisms associated with lymphocyte activation; PHA, though a potent non-specific lymphocyte activator, induced little enzyme activity in cells from any of the disease groups. Thus induction of 2', 5'-A synthetase in lymphocytes is a potential means of screening putatively infected cells for low levels of interferon release.

Minor differences were observed in the ability of IFN- $\alpha$  to induce enzyme in lymphocytes from patients with different diseases. However, this may reflect the extent to which the blood concentration of responsive lymphocytes is affected by these diseases. Moreover, since the enzyme concentration in unstimulated cells also varied, impoverished responses to in vitro challenge with IFN- $\alpha$  may be explained by their relative refractoriness to further stimulation. Defective lymphocyte production of interferons has been reported in SLE,<sup>12</sup> and there

has been considerable speculation about possible defects in the ability of different mononuclear populations in this disease to produce or respond to interferons. Since SLE lymphocytes produce 2', 5'-A synthetase both spontaneously and in response to IFN- $\alpha$ , it is unlikely that there are gross defects of this kind.

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*Ann Rheum Dis* 1986 45: 220-224

doi: 10.1136/ard.45.3.220

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