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(2′-5′) oligo A synthetase in human polymorphonuclear cells increased activity in interferon treatment and in viral infections

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SUMMARY

The interferon (IFN)-induced enzyme 2-5A synthetase was found in human peripheral blood polymorphonuclear cells (PMNL). The average enzyme activity in a group of 15 patients with various viral infections was significantly higher (25-fold) than in healthy individuals. Eight patients with multiple sclerosis and six patients with bacterial infections were found to have normal 2-5A synthetase levels in the PMNL. Relationship of PMNL 2-5A synthetase levels to IFN was confirmed by finding enzyme increases in PMNL incubated *in vitro* with IFN, as well as in patients undergoing IFN therapy. These findings suggest that in PMNL, as in other cells, the level of 2-5A synthetase can be regulated by IFN and can be increased as a result of IFN information in diseases.

Keywords 2-5A synthetase interferon polymorphonuclear cells viral infections multiple sclerosis

INTRODUCTION

One of the most sensitive biochemical markers for the response of cells to interferon (IFN) is an increase in the level of the enzyme (2′-5′) oligo A (2-5A) synthetase. The enzyme, which is ubiquitously found in cells of various types, increases within a few hours of treating cells by IFN (Roberts *et al.*, 1976; Kerr, Brown & Hovanessian, 1977), possibly as part of the changes which make the IFN treated cell resistant to viral infections (Knight *et al.*, 1980). It was found that the enzyme, in the presence of dsRNA, polymerizes ATP to form oligonucleotides which activate a ribonuclease, thus enhancing mRNA degradation and impairing viral protein synthesis (Revel, 1979). Our recent studies, as well as studies by others, suggest that 2-5A synthetase can serve as a marker not only for the *in vitro* response of cells to IFN, but also for a sensitive monitoring of the IFN system *in vivo* (Krishnan & Baglioni, 1980; Schattner *et al.*, 1981a, 1981b, 1982; Williams *et al.*, 1982; Preble *et al.*, 1983). Using a simplified assay for 2-5A synthetase (Merlin, Revel & Wallach, 1981), we have quantified its level in the peripheral blood mononuclear cells (PBMC) and found marked increases during the course of experimental viral infections in mice as well as in human with different viral diseases or under IFN therapy (Schattner *et al.*, 1981a, 1981b, 1982).

The aim of the present study was to examine the 2-5A synthetase system for the first time in human polymorphonuclear cells (PMNL). Although the effect of IFN on granulocytes has not been studied as extensively as its effects on lymphocytes and macrophages, there are clear indications that

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PMNL do response to IFN (Szigeti *et al.*, 1980; Hokland & Berg, 1981; Degre *et al.*, 1981). This was strongly confirmed by the finding of IFN related increases in 2-5A synthetase in PMNL. Thus, granulocytes can also be conveniently examined for IFN-induced changes, a method which may lead to a better understanding of their role in anti-viral defences.

MATERIALS AND METHODS

Isolation of cells and extraction. Mononuclear cells were separated from diluted, heparinized blood by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals) and counted in a haemocytometer. Cells were then pelleted in an Eppendorf microtest tube and suspended at a concentration of 10^7 viable cells/ml in lysis buffer containing 20 mM Hepes buffer pH 7·5, 5 mM $MgCl_2$, 120 mM KCl, 7 mM dithiothreitol, 10% (vol./vol.) glycerol and 0·5% Nonidet P-40 (NP-40). Lysis was carried out at 4°C and the nuclei removed by centrifugation for 6 min at 8,000*g*. The extract was either used immediately or stored at −70°C until assayed.

For PMNL isolation, the cells at the bottom of the Ficoll-Hypaque gradient were resuspended in one volume of 0·5 M NaCl. A 0.25 volume of 6T (wt/vol.) Dextran (T250, Pharmacia Fine Chemicals) in 0·15 M NaCl was added and the erythrocytes sedimented at 1*g* for 30 min at room temperature. The PMNL in the supernatant were centrifuged at 250*g* for 10 min and the cell pellet suspended in 34 mM NaCl for 45 s to lyse residual erythrocytes. Isotonicity was then restored with an equal volume of 0·27 M NaCl, the cells centrifuged for 10 min at 250*g*, washed twice in PBS, counted, and extracted as for mononuclear cells above. Differential counts of the PMNL preparations were performed following Giemsa staining. In repeated analyses, the content of mononuclear cells in the PMNL preparation was found never to exceed 3% of the cells.

In vitro *incubation of PMNL and PBMC with IFN.* PMNL or PBMCs suspended in RPMI 1640 with 10% heat-inactivated fetal calf serum in concentrations of 1×10^7 cell/ml were incubated for 18 h at 37°C in a humidified 5% CO_2 atmosphere with or without IFN-β (1,000 u/ml). The cells were then washed twice with PBS and extracted as described for PBMC. Cell viability before extraction as assessed by trypan blue exclusion was always higher than 94% for PBMC and higher than 80% for PMNL. The presence of IFN in the cultures had no adverse effect on cell viability. IFN-β was induced in cultures of foreskin fibroblasts by poly(rI):(rC) and partially purified to 10^7 units/mg protein by chromatography on carboxymethyl sepharose.

Enzyme assay. As detailed previously (Merlin *et al.*, 1981; Schattner *et al.*, 1981a, 1981b), 0·01 ml cell extract was mixed with 0·025 ml poly(rI):(rC) agarose beads (PL-Biochemicals) and incubated for 15 min at 30°C. To remove proteins which did not bind to poly(rI):(rC) the beads were washed with 1 ml of a solution containing 10 mH Hepes buffer, pH 7·5, 50 mM KCl, 5 mM $MgCl_2$, 7 mM dithiothreitol and 20% glycerol. They were then resuspended in 0·01 ml reaction mixture containing 10 mM Hepes buffer, pH 7·5, 5 mM $MgCl_2$, 7 mM dithiothrietol, 10% (vol./vol.) glycerol, 2·5 mM (^{32}P)-α-ATP (0·3 Ci/mmol). 10 mM creatine phosphate, 3 mg/ml creatine kinase and 40 μg/ml poly(rI):(rC). After incubation for 14–20 h at 30°C, 1 unit of bacterial phosphatase in 0·01 ml 140 mM Tris base was added and further incubated for 1 h at 37 °C. Two hundredths of a millilitre of water was then added, the beads removed by centrifugation and 0·01 ml of the supernatant was applied on a 0·3 ml alumina column (Acid WAI, Sigma) in 1 M glycine-HCl buffer pH2. A total of 3 ml such buffer was passed through the column, collected in scintillation vials and counted in the ^{3}H-channel of a Tri-Carb counter. In each series of assays, three samples from healthy individuals were included as standards.

Patients. 2-5A synthetase activity in PMNL was determined in blood samples from 15 consenting volunteers and in 15 patients with viral diseases. The patients were usually hospitalized and the diagnosis confirmed by serology in two-thirds of the cases (Table 1). In addition, six patients with bacterial diseases, eight patients with multiple sclerosis (MS) and some patients with other pathological conditions were also examined. Six of the patients with MS (five females, three males, mean age 34) had active disease and were examined during attacks, before initiation of ACTH treatment, and two patients were in remission. Changes in 2-5A synthetase in PMNL were also monitored in two patients undergoing IFN therapy. The first, an infant who died of fulminant

Table 1. 2-5A synthetase and serum IFN levels in polymorphonuclear cells (PMNL) and mononuclear cells (PBMC) of 15 patients with viral infections

		(2′-5′) oligo A synthetase		
		PMNL	PBMC	PMNL/PBMC
Diagnosis	Serum IFN	pmole ATP incorporated into $(A2'p)_nA/10^5$ cells/h		
Average in 15 healthy subjects	0	25	125	0·2
Encephalitis†	0	750	325	2·3
Encephalitis	64	360	390	0·9
Encephalitis	64	715	415	1·7
Encephalitis	0	40	420	0·1
Hepatitis A	16	120	715	0·2
Hepatitis, non-A non-B	250	1,500	455	3·3
HBsAg+(blood)	ND*	350	480	0·7
Infectious mononucleosis	125	1,820	625	2·9
Infectious mononucleosis	250	90	765	0·1
CMV Infection	64	1,520	350	4·3
Viral pneumonia	250	1,300	430	3·0
Laryngeal papillomatosis	0	170	350	0·5
Viral enteritis	ND	120	610	0·2
Viral upper respiratory infection	ND	300	390	0·8
Viral upper respiratory infection	0	80	235	0·3
Average in viral infections	90	615	464	1·3

* ND = not determined.

† Of the four encephalitis patients, two had herpes virus encephalitis, one had measles-encephalitis and in one case the virus was not identified.

Table 2. 2-5A synthetase in PMNL and PBMC before and after IFN treatment

		PMNL			PBMC		
IFN treatment	Samples	Control	IFN	IFN/control	Control	IFN	IFN/control
In vitro†	I	65*	455	7	90	570	6·3
	II	40	505	12·6	115	440	3·8
	III	125	1220	9·8	130	360	2·8
	IV	15	290	19·3	95	535	5·6
In vivo‡	V	25	515	21	125	600	4·8
	VI	170	435–495	2·6–2·9	350	585–750	1·7–2·2

* pmoles ATP incorporated in 1 h into $(A2'p)_nA$ by extract from 10^5 cells.

† Cells of four healthy individuals were incubated for 18 h with or without 1,000 u IFN-β/ml.

‡ Patients undergoing therapy with IFN. Activities right before and 24 h after IFN treatment are given. Sample V was taken 24 h after the second injection of 1×10^6 units IFN-α. The results given for sample VI are the range of activities observed in six repeated determinations during 1 month of treatment by repeated injections of $1{\cdot}5 \times 10^6$ units IFN-α (see Materials & Methods).

hepatitis, received two intramuscular injections of IFN-α (1×10^6 units), and the second, a child with laryngeal papillomatosis, has been receiving $1{\cdot}5 \times 10^6$ u/48 h for more than 6 months, with good clinical results (Table 2, samples V & VI, respectively).

RESULTS

2-5A synthetase in PMNL from healthy subjects

Significant activity of 2-5A synthetase could be detected in extracts of PMNL. The mean activity in a group of 15 healthy individuals was $24{\cdot}6 \pm 28{\cdot}6$ (s.d.) pmoles ATP incorporated into $(A2'p)_nA/10^5$ cells/h, only about a fifth of the average activity normally found in PBMC. Repeated analysis of the purified PMNL showed that contaminating PBMC usually constituted less than 1%, and never exceeded 3% of the cells in the preparation. Therefore, 2-5A synthetase activity found in these preparations could not be attributed to contaminating PBMC. As opposed to the enzyme level in PBMC, which is relatively constant in the healthy population (Schattner *et al.*, 1981b), we found that the activity in PMNL varied considerably.

To determine the degree of responsiveness of the PMNL 2-5A synthetase to IFN, we incubated cells from four healthy donors for 18 h with IFN and found a dramatic increase of the enzyme level in all cases (Table 2). In contrast, the synthetase level in PMNL cultured in the presence of saline only, was not increased. We then measured the level of enzyme in cells from patients undergoing IFN therapy and found that it was also sharply increased. In one of the cases (VI, Table 2) the increase of synthetase activity was maintained during prolonged IFN treatment (1 month). The extent of the IFN-induced increase in 2-5A synthetase activity of the PMNL in both the *in vitro* experiment and following IFN therapy, was higher than that induced simultaneously in the PBMC (Table 2).

Increased PMNL 2-5A synthetase in viral infections

While the 2-5A synthetase levels in PMNL of patients with bacterial infections or MS were comparable to those of the healthy controls, a significant increase in enzyme activity could be detected in most cases of viral infections (Fig. 1). The mean activity in this group was 25-fold higher than that of the healthy controls (615 *vs* 25 pmoles/10^5 cells/h). In several of these patients the activity in the PMNL was significantly higher than the level of the enzyme in PBMC (Table 1). A comparison of the changes in PMNL to the level of IFN, detected in the serum, showed only partial correlation. Interestingly, the synthetase was found to be increased in four cases of viral infections

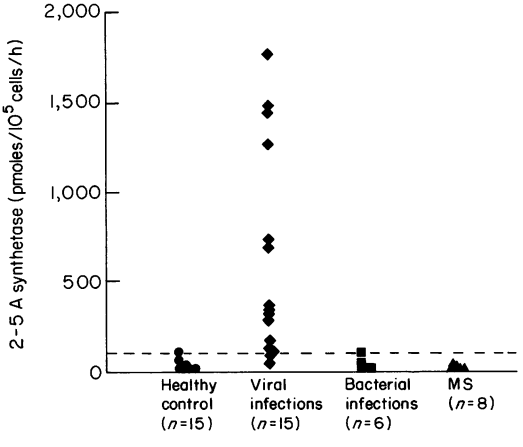

Fig. 1. 2-5A synthetase levels in PMNL of healthy controls and patients with viral infections, bacterial infections or multiple sclerosis. The broken line shows the mean synthetase activity in normal PBMC.

that were serum IFN negative (Table 1). No correlation could be found between the enzyme level and the relative proportion of eosinophils in the PMNL population (not shown). Marked eosinopenia was found in two patients with viral infections and in one donor of the *in vitro* study. The fact that very high levels of PMNL 2-5A synthetase were still obtained may indicate, though not with certainty, that the enzyme is not eosinophil derived.

Increases of the synthetase level in PMNL are not necessarily restricted to viral infections. Initial characterization of other situations in which the level of the synthetase in PMNL might be increased showed a significant increase of the enzyme level in a few cases of various unrelated pathological conditions including dermatomyositis, nephrotic syndrome in children and Henoch–Schönlein purpura (not shown). Thus, 2-5A synthetase determination in PMNL could detect activation of the IFN system in diverse conditions of uncertain aetiology, as well as rule out such a state in the patients with MS we examined.

DISCUSSION

Our previous studies on the variation of 2-5A synthetase activity have demonstrated that IFN can induce an increase in the level of the enzyme in circulating cells. They have also indicated that changes in the level of the synthetase may sometimes give a more sensitive indication for the existence of IFN in the body than that obtained by measuring the level of serum IFN itself (Schattner *et al.*, 1981a, 1981b, 1982; Wallach *et al.*, 1982). This is probably due to the fact that cellular changes, induced by IFN, persist for quite a time after removal of IFN from the cell. A particular advantage in the use of circulating lymphocytes for that purpose is that these cells may be exposed to locally formed IFN which may not be detectable in serum.

In this study we show that 2-5A synthetase activity is detectable also in PMNL and that this activity can be increased by IFN. Moreover, the extent of enzyme increase in these cells can be significantly larger than that observed in the PBMC, since the basal activity in PMNL is lower, and levels following exposure to IFN may be as high as in the PBMC. On the other hand, it is expected that the duration of enzymes increase in the PMNL fraction of the leucocytes, following *in vivo* formation of IFN, will be shorter than in the PBMC, since the survival time of the former is much shorter than that of recirculating lymphocytes (Ottenson, 1954). The considerable variation of enzyme activity noted in different individuals may be due to constitutive differences, or possibly to the occasional formation of low levels of IFN in the absence of any symptoms, a point which may be clarified by future kinetic studies. The high 2-5A synthetase levels in PMNL which we demonstrated in many patients with viral infections, are in line with the observed changes induced by exogenous IFN, whether administered *in vitro* or *in vivo* (Table 2), and can be confidently related to the effect of endogenous IFN. Furthermore, we have repeated that 2-5A synthetase determination in four of the patients 10–14 days after complete recovery from their viral diseases and found levels similar to those of healthy individuals. In conrast, when exposure of the PMNL to IFN was prolonged (VI, Table 2), enzyme levels remained continuously elevated for over a month.

The fact that PMNL show a marked increase of 2-5A synthetase following exposure to IFN is consistent with prior studies which suggested that functions of PMNL can be affected by IFN (Szigeti *et al.*, 1980; Hokland & Berg, 1981; Degre *et al.*, 1981). There are several indications that PMNL can participate in the response of the immune system to viral infections. It has been observed, for example, that PMNL concentrate in areas infected with polio virus (Allison, 1974) or herpes simplex virus (McSorley *et al.*, 1974), and that they can suppress viral infection by mediating antibody-dependent cellular cytoxicity against virus infected cells (Rouse *et al.*, 1977; Oleske *et al.*, 1977). PMNL have been reported also to produce an IFN like mediator when coming in contact with virus infected cell preparations (Rouse, Babiuk & Henson, 1980). Further studies on proteins like the (2′-5′) oligo A synthetase which are induced by IFN in PMNL and on functions which they regulate in these cells, may be of great importance for promoting the understanding of the role of PMNL in the defense against diseases.

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