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Human acid- and thermolabile α-interferon-like substance: selective reactivity with a monoclonal antibody

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Summary

An acid- and thermolabile α-interferon-like substance, designated AL-IFN-α, has been found in non-processed normal human leukocyte IFN preparations as well as sera from patients with autoimmune or other chronic diseases. Little is known about origin, production and biological activity of these IFN activities. Monoclonal antibodies were obtained which proved highly selective in neutralizing AL-IFN-α in both anti-proliferative and antiviral tests. While the monoclonal antibodies were strict specific, polyclonal antibodies against various interferons showed less specificity in these tests. The results suggest that AL-IFN-α represents an antigenically distinct IFN-α subtype or, alternatively, a new lymphokine with anti-proliferative and antiviral activity.

Interferon-like substance; Monoclonal antibody; Polyclonal antibody; Autoimmune disease

Introduction

Several chronic diseases may be accompanied by temporal or intermittent presence of interferon (IFN) activity in the patients' plasma. This is the case for the human autoimmune diseases (Hooks et al., 1979; Waschke and Diezel, 1984; Rovenský et al., 1984), AIDS (Byster et al., 1983) and for Down's syndrome (Funa et al., 1984). The IFN activity found in the sera of the majority of the patients proved to be acid- and thermolabile. However, in contrast to acid- and thermolabile IFN-

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γ, this IFN species was neutralized by anti-IFN-α sera. It also exerted antiviral activity in Madin-Darby bovine kidney cells, thus indirectly supporting its α-type character (Preble et al., 1982; Borecký et al., 1986a).

Recently, an acid- and thermolabile species of IFN was found to be present, in various proportions, in freshly prepared human leukocyte IFN preparations (Chadha et al., 1985; Borecký et al., 1986b). Due to its acid- and thermolability and, the low amounts circulating in the blood, the precise characterization of these IFN-like substances has proved difficult.

In this study we report on a monoclonal antibody which reacts selectively with an acid- and thermolabile IFN-like preparation from freshly induced human blood leukocytes and cross-reacts with interferon-like substances present in the sera of patients with psoriasis or systemic lupus erythematosus (SLE).

Material and Methods

Interferon (IFN)

The following preparations of human IFNs were used: acid- and thermostable human leukocyte IFN-α (S-IFN-α) with an antiviral activity of 2.5×10^6 units/ml; acid- and thermolabile IFN-α (AL-IFN-α) with an antiviral activity of 1.28×10^4 units/ml. These preparations were provided by Dr. N. Fuchsberger from this Institute. The freshly obtained human leukocyte suspension (10^6 cells/ml in Eagle's basal medium complemented with 10% inactivated calf serum) was freed of erythrocytes by treatment with 0.83% ammonium chloride. The cells were then infected with Newcastle disease virus (NDV-B1) by adding 0.1 ml of virus suspension (approximately 512 hemagglutinating units) to 1 ml of leukocyte suspension (10^6 cells/ml). After incubation at 37°C for 18 h, the cells were disrupted by one cycle of freezing and thawing and centrifuged at 3000 rpm for 15 min. The supernatant was then centrifuged at 30000 rpm for 90 min in a MSE (Rotor 1010) centrifuge and used subsequently for immunization and other tests. Its antiviral titer was 1:12800 which dropped to 1:256 after acidic treatment, pH 2, for 3 days. The antiviral activity of both preparations was standardized with an NIH IFN-α standard (Ga-23-902-530).

Human fibroblast IFN-β (BM 532, Lot G-8510) with an antiviral activity of 10^6 units/ml was a gift of S. Kobayashi, Toray Ind., Japan, while the human leukocyte IFN-γ with an antiviral titer of 10^6 units/ml was a gift of Finnish Red Cross, Helsinki, through courtesy of Dr. K. Cantell. These preparations were standardized with NIH standards G-023-901-527 (for IFN-β) and Gg-23-901-30 (for IFN-γ).

The recombinant IFN-α subtypes: IFN-α1 and IFN-α2 were a gift of Dr. G. Bodo (Boehringer, Vienna) and the recombinant IFN-αN was obtained from Dr. E. Gren, Latvian Academy of Sciences, Riga.

In comparative tests, sera from patients with systemic lupus erythematosus (SLE) or psoriasis showing antiviral and other interferon-like activities were used as antigens.

Antisera

Polyclonal antiserum against AL-IFN-α was obtained after immunization of BALB/c mice (details will be published elsewhere). Sheep antisera to human leukocyte IFN-α (G-026-502-568), to human fibroblast IFN-β (G-028-501-568) and rabbit antiserum to human IFN-γ (G-034-501-565) were obtained through the courtesy of Dr. C.A. Laughlin from the National Institutes of Health, Bethesda, MD, USA. In some tests a polyclonal sheep antiserum to human leukocyte IFN-α produced in this institute was also employed.

Antiserum against Newcastle disease virus (NDV-B1) was prepared by immunization of BALB/c mice with four doses of NDV-protein (300 μg/mouse) intraperitoneally at 2-week intervals. Its titer by ELISA was 1:16000.

Monoclonal antibodies (mAb)

The following monoclonal antibody preparations were used: mAb to human AL-IFN-α: T-18 and T-19 (IgM type) and T-24 (IgG1 type), mAb to human S-IFN-α: 60/4 (IgG1 type) (Novák et al., 1986), mAb to human IFN-β: B13 (IgG1) (Kontsek et al., 1989), mAb to human IFN-γ-A8 (IgG1) (Kontsek et al., 1988). The mAbs against S-IFN-α, IFN-β and IFN-γ were used in ascitic form. Those of IgG1 subtype were purified by protein A affinity chromatography, while the mAb of IgM type were precipitated with 50% ammonium sulfate. The concentration of protein in the mAb preparations was determined by the method of Lowry. The amount of mAb in the preparations was determined by ELISA (Kontsek et al., in preparation).

Interferon assay

Antiviral activity of the IFN preparations was determined by reduction of the cytopathic effect of vesicular stomatitis virus (VSV) in primary human embryonic fibroblasts in microtiter plates (Hardmuth, Koh-i-noor, Czechoslovakia). The assay was calibrated against NIH reference IFN standards (see above).

Neutralization of antiproliferative activity of IFN

The ability of antibodies to neutralize the antiproliferation activity of IFN was assayed on HL-60 cells of myelocytic origin as described elsewhere (Kontsek et al., 1988). Briefly, IFN at 200 (or an indicated number of) units in 0.05 ml volume was mixed with an equal volume of antibody in a microwell and, subsequently, 2×10^4 HL-60 cells in 0.1 ml were added to the mixture. After incubation for 72 h at 37°C, the number of cells/well were counted using a Burker chamber and the antiproliferative effect was determined. In this cell system, 200 units of IFN per 0.05 ml reduced the number of HL-60 cells to 70% (68–76%) of the control cell number. IFN-γ, due to its stronger antiproliferative effect was used at a concentration of 10 units/0.05 ml. Each IFN-antibody combination was assayed in quad-

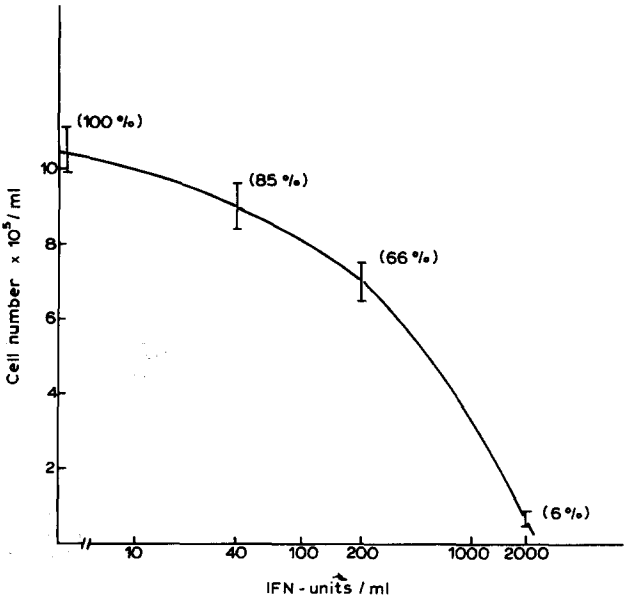

Fig. 1. Dose-response curve for AL-IFN-α in antiproliferative assay; y-axis: cell number after 72 h at 37°C. x-axis: IFN units/0.05 ml.

ruplicate. The results, i.e. the cell number/ml ± standard deviation ($\bar{x} \pm SD$), are expressed as means for 4 wells. Neutralization of antiproliferative activity by 100% corresponds to the mean number of cells incubated in absence of IFN, while 0% neutralization corresponds to the cell number after incubation with IFN in the absence of antiserum. At the concentration used, IFN resulted in statistically significant reduction of the number of cells. The neutralization titer was the highest dilution of antiserum, or, the minimal concentration of mAb in μg/ml, which inhibited the antiproliferative activity by more than 90%. The dose-response curve for inhibition of proliferation of HL-60 cells by AL-IFN-α is shown in Fig. 1.

Neutralization of antiviral activity of IFN

A slight modification of the procedure of Preble et al. (1982) was utilized. Serial dilutions of the IFN preparation, starting usually with dilution 1:3, in 0.5 ml, were mixed with a constant dilution of polyclonal antibody or purified preparation of mAb at the indicated amounts in a volume of 0.05 ml, and added to diploid human fibroblasts in microtiter plates. The neutralization titer corresponded to the maximal concentration of IFN in units/ml that was reduced to one unit by antibody. Hybridomas secreting mAbs which neutralized more than three units of IFN activity were considered positive.

Results

The human leukocyte IFN-α preparation used as antigen for obtaining monoclonal antibodies against the human acid- and thermolabile α-IFN-like substance (AL-IFN-α) showed a high inhibitory activity against the cytopathic effect of VSV in primary human embryonic lung fibroblasts. The antiviral titer was 1:12 800 per ml; it decreased to 1:256 after acid treatment, pH 2, for 3 days. This suggests that the AL-IFN-α component may occasionally represent more than 90% of antiviral activity in virus-induced leukocyte IFN preparations. The preparation also showed antiviral activity in MDBK cells, while no antiviral activity could be detected in mouse cells (Borecký et al., 1986). This indirectly supports the α character of AL-IFN.

The properties of 3 of the 6 monoclonal antibodies against AL-IFN-α are shown in Table 1. In most of tests presented in this article, the monoclonal antibody designated T-18 (IgM type) was used either as ascitic fluid or as culture supernatant from hybridomas. From Table 1 it is clear that the mAb against AL-IFN-α neutralized both the antiviral and the antiproliferative activity of AL-IFN-α. This activity was correlated with the relative Ig concentration present in the preparation.

The cross-reactivity of AL-IFN-α with the IFN-like substances found in SLE and psoriatic patients is shown in Table 2. While the polyclonal anti-S-IFN-α-serum, which was produced in our Institute, showed cross-reactivity with the AL- and autoimmune – SLE or psoriatic – IFN samples in the (antiviral) neutralization tests, the monoclonal anti-S-IFN-α antibody clearly distinguished S- from AL-IFN (Tables 3, 4). Several IFN-positive SLE sera were tested with essentially similar results (not shown).

TABLE 1

Characterization of monoclonal antibodies neutralizing AL-IFN-α

Culture supernatant	ELISA		Neutralization of		
	Ig-type[a]	Ig-concentration[a]	Antiviral activity[b] (units of AL-IFN-α neutralized per ml)	Antiproliferative activity[c] (cell number × 10^4 per ml)	
				$\bar{x} \pm SD$	Control (%)
T-18	M	1:1024	24	70.3 ± 6.2	100
T-19	M	1:256	12	67.2 ± 4.4	96
T-24	G1	1:64	3	52.4 ± 4.4	75
NSO[d]	–	–	–	49.3 ± 3.2	70
NSO[e]	–	–	control without IFN	70.5 ± 4.6	100

[a]Type and relative concentration of Ig in hybridoma culture supernatant, determined in ELISA with appropriate indicator sera.

[b]The neutralizing titer was the maximal concentration of IFN units/ml that was reduced by antibody to 1 unit.

[c]Reduction of antiproliferative activity of 200 antiviral units per 0.05 ml after mixing AL-IFN-α with an equal volume (0.05 ml) of hybridoma cell supernatant. The experiment was done in triplicate. Average values ± standard deviation (SD) are listed.

[d,e]Culture supernatant from Ig-non-secreting NSO myeloma cells in the presence[d] or absence[e] of AL-IFN-α.

TABLE 2

Cross-reactivity of interferon-like preparations from leukocytes or autoimmune sera in antiviral tests

Interferon-like substance	Antibodies				
	Polyclonal against			Monoclonal against	
	S-IFN-α	IFN-β	IFN-γ	S-IFN-α	AL-IFN-α
AL-IFN-α	81/9 (+)[c]	27/27 (−)	27/27 (−)	81/27-81[d]	81/9 (+)
SLE-IFN[b]	32/8 (+)	32/32 (−)	32/32 (−)	ND[e]	32/<8 (+)
Psoriatic IFN[b]	32/<8 (+)	ND	32/16 (−)	ND	32/<8 (+)

[a]In this test, the AL-IFN-α was diluted to contain 27 to 81 antiviral units per 0.05 ml to adjust the antiviral potency of AL-IFN-α to antiviral activities usually found in SLE and psoriatic sera.
[b]Patients' sera with antiviral activity as indicated.
[c]The neutralization of antiviral activity (against VSV) in human lung fibroblast cells was considered positive (+) if the decrease of antiviral activity in the presence of antisera was more than 3-fold and negative (−), if the decrease was less than 3-fold (Preble et al., 1982).
[d]Range in various experiments.
[e]ND, not done.

Occasionally also anti-IFN-γ sera were found to contain antibodies against the AL-IFN-α (Table 3). As shown in Table 4, the purified anti-AL-IFN-α monoclonal antibody (T-18) exerted a relatively high (antiproliferative) neutralizing activity against AL-IFN-α (>90% reduction in activity with 1 μg of antibody), while it was ineffective against S-IFN-α, natural IFN-α, -β and -γ, and recombinant IFN subtype α1, α2 and αN even at concentrations exceeding 10 μg of antibody/ml.

Since NDV was used as the inducer of IFN in human leukocytes, an anti-NDV serum was also tested for its reactivity with AL-IFN-α. However, such serum effected only 7% neutralization of the antiproliferative effect when used at various concentrations (data not shown). Moreover, the occasional finding of anti-AL-IFN-α antibodies in anti-IFN-γ sera (Table 3), which were obtained using IFN-γ preparations induced by mitogens argues against the presence of NDV constituents in the AL-IFN-α samples. Finally, neither polyclonal nor monoclonal (A8) anti-IFN-γ antibodies showed an AL-IFN-α neutralizing activity (Fig. 2).

TABLE 3

Lack of cross-reactivity of S- and AL-IFN-α with their respective antibodies in antiproliferative tests

Human leukocyte IFN	Antiproliferative activity[a] of 200 units/ml (cell number in percentage of control)	Neutralization[b] of antiproliferative activity by antibodies				
		Monoclonal against (μg/ml)			Polyclonal against (dilutions)	
		AL-IFN-α	S-IFN-α	AL-IFN-α	S-IFN-α	IFN-γ
AL-IFN-α	73	1.0	>10.0	10^{-3}	$<2.5 \times 10^{-1}$	5×10^{-1}
S-IFN-α	68	>10.0	2.0	10^{-2}	10^{-3}	$<10^{-1}$

[a]Number of cells 72 h after mixing the IFN sample in 0.05 ml with 2×10^4 HL-60 cells in 0.1 ml.
[b]Concentrations of monoclonal antibodies in μg/ml and dilutions of polyclonal antisera which reduced the antiproliferative activity by >95% of the IFN preparations.

TABLE 4

Selective neutralization of the anti-proliferative activity of AL-IFN-α with anti-AL IFN-α monoclonal antibody T-18 in HL-60 cells

IFN 200 units/ml	Concentration of cells[a] × 10^4/ml				Significance of difference (t) Control vs IFN-treated cells P (N = 8)	Neutralizing capacity of monoclonal antibody T-18 (μg/ml)[b]
	$\bar{x}$	±	SD	%		
AL-IFN-α	50.7	±	5.4	73	<0.0005	1.5
S-IFN-α (Cell-tech)	47.5	±	3.5	68	<0.0005	>10.0
r-IFN-α1 (Boeh-ringer)	52.0	±	5.2	75	<0.0005	>10.0
r-IFN-α2 (Boeh-ringer)	47.3	±	5.0	68	<0.0005	>10.0
r-IFN-αN (Riga)	49.2	±	2.1	71	<0.0005	>10.0
IFN-β (Toray)	50.2	±	2.7	72	<0.0005	>10.0
IFN-γ (Hel-sinki)[c]	53.0	±	3.3	76	<0.0005	>10.0
Control (no IFN)	69.6	±	4.6	100		

[a]Antiproliferative activity of IFN preparations after 72 h of incubation (Number of cells ± SD and % of control (untreated) cells. 2 × 10^4 cells per well seeded.

[b]Concentration of T-18 in μg/ml which neutralizes the antiproliferative activity of 200 units of IFN by 90%.

[c]10 IFN-γ units per 0.05 ml; $\bar{x}$, average values ± standard deviation (SD); t, Student's t-test; P, significance limit.

Discussion

IFNs found in the sera of patients with autoimmune diseases are generally considered to be of the α type. In previous studies, polyclonal antisera were used to determine the antigenic type of these IFNs and further characterization has been hampered both by the relatively low concentrations of IFN found and its acid- and thermolability. An acid- and thermolabile IFN component has also been found in crude normal human leukocyte IFN preparations (Chadha, 1985; Borecký et al., 1986, 1987). We used such a crude leukocyte IFN-α preparation to obtain antisera against AL-IFN-α in BALB/c mice. The preparation consisted of acid-labile and acid-stable IFN molecular populations in a 50:1 proportion. In this way, both polyclonal antiserum and, after fusion of immune spleen cells with NSO myeloma cells, monoclonal antibodies were obtained. Several hybridomas showed the capacity to produce mAbs distinguishing the acid- and thermolabile IFN-like activity (AL-IFN-α) from the 'classical' acid- and thermostable S-IFN-α type, the recombinant subtypes (α1, α2, βN) and the natural and recombinant IL-2 (data not shown). These mAbs also reacted with acid- and thermolabile IFNs found in lupus and psoriatic patients (Table 2). The distinct antigenicity of AL-IFN-α is also supported by the presence of AL-IFN-α in an IFN-γ preparation that was not induced by virus. AL-IFN-α also exhibits antiviral activity in MDBK cells, which indirectly supports its α-type character.

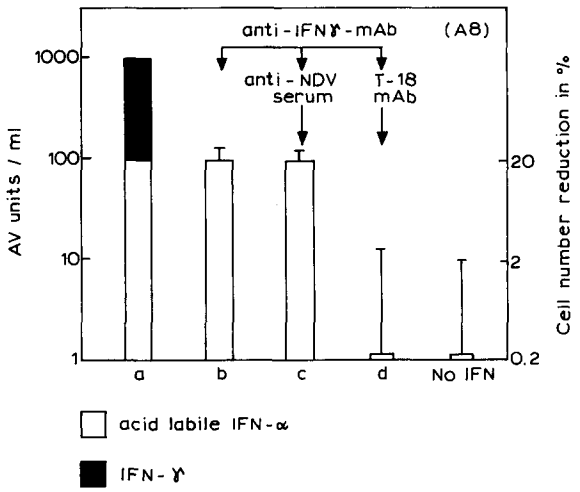

Fig. 2. Detection of AL-IFN-α component in IFN-γ preparation with anti-AL-IFN-α monoclonal antibodies. Right ordinate: antiproliferative activity of IFN preparation tested (% reduction in cell number). Logarithmic scale. Left ordinate: IFN concentration in antiviral units per 0.05 ml. Abscissa: (a) The IFN preparation consisting of 900 antiviral units of IFN-γ and 100 antiviral units of AL-IFN-α reduced the growth of HL-60 cells by more than 40%. (b) Addition of anti-IFN-γ mAb (A8) inactivates the antiproliferative effect of 900 units of IFN-γ while the antiproliferative effect of 100 units of AL-IFN-α is not affected. (c) The inactivating effect of non-neutralized IFN (AL-IFN-α) is not enhanced by anti-NDV serum. (d) Total inactivation of antiproliferative effect of IFN preparation after addition of anti-IFN-γ (A8) plus anti-AL-IFN-α (T-18). The standard deviations were obtained within 12 parallel observations. They are given as log values of reduced cell numbers in per cent. The SD for the bar indicated as 'No IFN' (less than 2% reduction of cell number) represents the variability in the number of control cells (100 ± 2%).

At present, little is known about the origin, mechanism of induction and biological properties of AL-IFN-α. After Sephadex G100 chromatography of normal leukocyte IFN-α preparations, AL-IFN-α can be found in the heavy 'A' peak (Borecký et al., 1986). This suggests a higher molecular weight for AL-IFN-α than for S-IFN-α. Due to its acid- and thermolability, AL-IFN-α may disappear from IFN preparations during processing and storage. However, if present in the IFN preparations, it may influence the quality of the polyclonal antisera raised.

The results of this study support the possibility that AL-IFNs present in stimulated human leukocyte preparations and, probably, in sera of autoimmune patients represent an antigenically different IFN subspecies or subtype. However, it cannot be excluded that AL-IFN-α represents a new lymphokine, distinct from the other lymphokines that have been described so far but sharing with them both antiviral and antiproliferative properties.

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