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INTERFERON SYSTEM IN PATIENTS WITH RHEUMATOID ARTHRITIS AND SCLERODERMIA SYSTEMATICA

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Summary. – In the blood of patients with rheumatoid arthritis and/or scleroderma systematica usually acid-labile interferon-alpha (IFN-alpha) was found. Blood leukocytes cannot be considered the source of its production as they spontaneously produce IFN-gamma identified with specific antiserum. Blood leukocytes of tested patients generated *in vitro* a reduced amount of staphylococcus enterotoxin A-induced IFN-gamma and virus-induced acid-labile IFN-alpha. This findings support the assumption of impaired functioning of T- and B-blood cells in autoimmune diseases. The production of Newcastle diseases virus-induced IFN-alpha and influenza virus-induced acid-stable IFN-alpha by patients' leukocytes has not been altered. Acid-labile IFN-alpha obtained from the blood of tested patients, IFN-gamma spontaneously generated by leukocytes *in vitro* and acid-labile IFN-alpha produced by leukocytes *in vitro* following induction with influenza virus show similar sensitivity to pH 2.0 and time patterns of the antiviral state development in human diploid fibroblast culture.

Key words: autoimmune diseases; acid-labile interferon-alpha; spontaneous gamma interferon-alpha

Introduction

Autoimmune diseases (AID) cover a number of complicated diseases insufficiently characterized in terms of their pathogenesis, and their aetiological agent. These diseases impair intercellular interactions resulting in generation of antibodies to autological antigens (Ricci *et al.*, 1988). Acid-labile IFN was detected in the blood of patients with AID (Skurkovich and Eremkina, 1975). Their findings were confirmed by other investigators showing that this IFN is of the alpha type (Hooks *et al.*, 1979; Preble *et al.*, 1982). Conceivably, the

functioning of the IFN system in these patients was impaired. IFN, as a rule, was detected in the blood of healthy individuals. On the other hand, the reduced production of IFN-alpha and IFN-gamma by AID patients leukocytes has been reported (Shiozawa *et al.*, 1988; Stolzenburg *et al.* 1988). Consequently, it appeared interesting to study the functioning of the IFN system in AID patients' with the aid of technique developed by us, namely to estimate the human leukocytes' capacity to produce *in vitro* influenza virus-induced acid-labile IFN-alpha.

Materials and Methods

Leukocytes. Heparinized blood of 10 patients with rheumatoid arthritis (RA), 10 patients with sclerodermia systematica (SDS) and 10 donors was used in all experiments. Blood leukocytes were isolated by erythrocytes lysis with cooled 0.83 % solution of ammonium chloride (in the ratio of 1:4) for 5–7 min. Following two washing, the leukocyte concentration was adjusted to 4×10^6 cells/ml in 2-fold Eagle's medium and supplemented with 10 % foetal calf serum, 600 μg/ml glutamine and 50 g/ml gentamycin.

IFN inducers. The following inducers were used: Newcastle diseases virus (NDV), H strain ($10^{9.5}$ EID_{50}/ml), influenza virus (IV), WSN strain (10^9 EID_{50}/ml) and staphylococcal enterotoxin A (SEA) obtained through the courtesy of Prof. Ju. V. Ezepchuk. The leukocyte suspension was incubated with NDV (1/10 volume) for 24 hr with subsequent acidification of the supernatant with 1N HCl to pH 2.0. On the 7th day the pH of medium was adjusted to neutral with 1 N NaOH. Acid-labile (IFN/AL) and acid-stable (IFN/AS) IFN-alpha were obtained by the procedure described previously (Scheglovitova *et al.*, 1990). After the incubation of leukocyte suspension with IV (1/10 volume) for 24 hr, a portion of the supernatant was treated with β-propiolactone (BPL) in the final dilution of 1:4000 at 4 °C for 24 hr. The rest was treated with 1 N HCl in the same way as the NDV-induced IFN. BPL-treated material contained acid-labile [INF/AL(IV)] and acid-stable [IFN/AS(IV)] IFN components; the material treated with acid at pH 2.0 contained only acid-stable component. After IFN induction with SEA (1 μg/ml) [(IFN-gamma (SEA)] the leukocyte suspension was incubated for 72 hr using the supernatant without additional treatment.

IFN titration and typing. IFN was tested in 96-well plates with 3 days-old monolayer of human diploid fibroblasts. Two-fold dilutions of the samples were applied into the wells and after 24 hr cultivation at 37 °C, 100 CPE_{50} of murine encephalomyocarditis virus was added to each well. The results were recorded after 24 hr cultivation at 37 °C. IFN titer was defined as the endpoint dilution inducing 50 % inhibition of the cytopathic effect of 100 CPE_{50} of the indicator virus. For IFN typing the antisera to human IFN-alpha and -gamma obtained through the courtesy of Dr. V. I. Iovlev were used. The mixture of 0.1 ml of tested material with 0.1 ml of the antiserum was incubated at 37 °C for 1 hr with subsequent determination of the residual antiviral activity by the routine procedure.

To assess the time course of antiviral state development 3 days-old monolayer of human diploid fibroblasts in the wells was exposed to 2-fold dilutions of the sample under study, and 100 CPE_{50} of the indicator virus was added 1–24 hr later. The results were evaluated routinely. The level of the antiviral state developed within 24 hr was defined as 100 %.

Results

Patients with chronic RA and/or SDS, aged 20–51, with the diseases duration varying from 1 to more than 11 years entered the study. First of all, the capacity of leukocytes of these patients to produce *in vitro* IFN/AL (IV) was studied. One part of supernatant of the leukocyte suspension exposed to IV after cultivation

for 24 hr was treated with BPL, while the other one was treated at pH 2.0. The test revealed the difference in the titers of BPL- and acid-treated materials. The BPL-treated samples contained the IFN/AL (IV) and indicated that the patients' leukocytes were able to produce IFN/AL following IV induction. Next we compared the capacity of RA and SDS patients and donors to produce IFN-alpha (NDV), IFN-gamma (SEA), IFN/AL (IV) and IFN/AS (IV) (Table 1). The IFN/AL (IV) production by leukocytes of RA and SDS patients proved to be lower as compared with the donors' leukocytes [5.7 ± 0.7, 5.4 ± 0.6 (patients)] and 7.28 ± 0.56 (control, respectively), the difference being statistically significant. The patients also appeared to produce lower IFN-gamma (SEA) level: 4.6 ± 0.6, 4.7 ± 0.4 (patients) and 6.85 ± 0.42 (control), respectively, the difference being also statistically significant. Leukocytes of patients with RA and SDS produced *in vitro* IFN-alpha (NDV) and IFN/AS (IV) in the same amount as did donors' leukocytes.

We studied also the time course of IFN/AL (IV) and IFN/AS (IV) production by leukocytes of RA and SDS patients and donors (Fig. 1). IFN/AL (IV) was detected in the culture media of donors' (control) leukocytes by 3 hr in the titer of 64 U/ml; its activity rised to 512 U/ml by 24 hr and remained at the same level by 72 hr (period observation). In the culture media of patients' leukocytes IFN appeared only by 6 hr in the titer of 16 U/ml, rising by 24 hr to 128 U/ml and persisting at the same level by 72 hr. IFN/AS (IV) was detected in the culture media of donors' leukocytes 6 hr after induction in the titer of 32 U/ml, which rised by 24 hr to 128 U/ml and dropped to 32 U/ml by 72 hr. IFN/AS (IV) was noted in the culture media of patients' induced leukocytes only after 24 hr cultivation in the titer of 32 U/ml, which fell to 8 U/ml by 72 hr. Consequently, time course of production of IFN/AL (IV) and IFN/AS (IV) by leukocytes of patients with RA and SDS and donors was similar. However, IFN/AL (IV) and IFN/AS (IV) were detected in the culture media of patients' leukocytes later than in those of donors. Decreased IFN/AS (IV) titers at later terms is accounted

Table 1. IFN production by leukocytes of donors and RA and SDS patients after induction with viruses and SEA

IFN type	IFN titer (log_2)				
	donors (n=10)	RA patients (n=10)	P	SDS patients (n=10)	P
IFN-alpha (NDV)	8.14 ± 0.28	8.26 ± 0.29	0.6	8.57 ± 0.3	0.1336
IFN-gamma (SEA)	6.58 ± 0.42	4.6 ± 0.6	0.0093	4.7 ± 0.4	0.0093
IFN/AL (IV)	7.28 ± 0.56	5.7 ± 0.7	0.05	5.4 ± 0.6	0.05
IFN/AS (IV)	3.0 ± 0.71	2.7 ± 0.48	0.6	3.1 ± 0.4	0.6

P = probability level from the test of significance

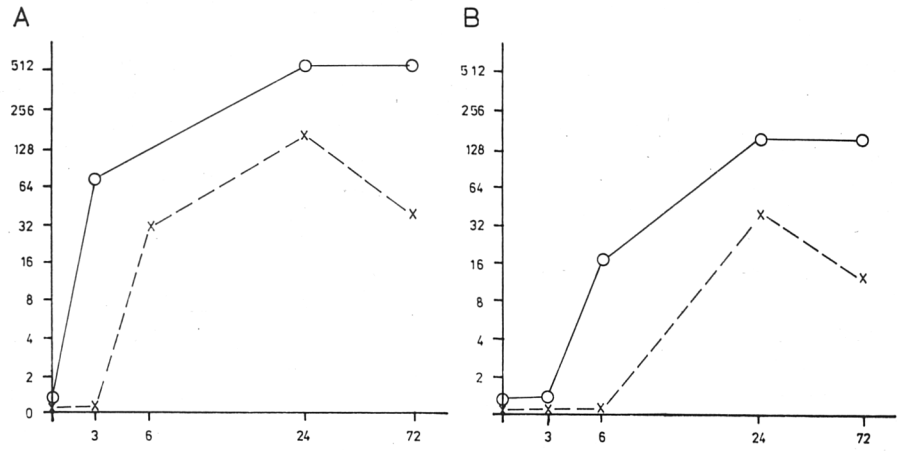

Fig. 1
Time course of IFN/AL (IV) and IFN/AS (IV) production by donors' and AID patients' leukocytes after induction with influenza virus
Abscissa: time (hr); ordinate: IFN titer (U/ml)
A: donors' leukocytes; B: AID patients' leukocytes
—O— IFN/AL (IV)
- - x - - IFN/AS (IV)

for by arising sensitivity to pH 2.0 by cultivation at 37 °C (Scheglovitova *et al.*, 1990).

Serum samples of RA and SDS patients contained IFN which was inactivated following acidification to pH 2.0. Blood leukocytes might represent a potential source of that IFN. To test this assumption, patients' leukocytes were cultivated at 37 °C for 24 hr without inducer. The culture fluid exhibited IFN termed "spontaneous" with antiviral activity that was lower than that of IFN found in blood. Following acid treatment at pH 2.0 the "spontaneous" IFN has completely lost its antiviral activity, as the serum IFN did (Table 2). The serum IFN as well as the IFN-alpha were completely neutralized by antisera to human IFN-alpha but not to IFN-gamma. The "spontaneous" IFN was completely neutralized by antisera to human IFN-gamma as was IFN-gamma. This indicates that the "spontaneous" IFN, in contrast to the serum IFN [IFN/AL (serum)], belongs to the gamma type of IFN [(IFN-gamma (spont)] and thus blood leukocytes of RA and SDS patients cannot be the source of IFN.

Various types of IFN obtained from patients were characterized in several tests (Table 3). They were incubated at 37 °C for 5 days with subsequent acid treatment at pH 2.0. The activity of IFN/AL (serum), IFN/AL (IV), IFN-alpha (NDV) and IFN-gamma (SEA) did not appear to be altered following the prolonged exposure at 37 °C. However, after treatment at pH 2.0 IFN/AL (IV), IFN/AL (serum) and IFN-gamma (SEA) were completely inactivated, whereas

Table 2. The effects of pH 2.0 and antisera to human IFN-alpha and IFN-gamma on the IFN activity

IFN type	Initial IFN titer (U/ml)	IFN titer (U/ml) after treatment with		
		pH 2.0	antisera to IFN-alpha	antisera to IFN-gamma
IFN/AL (serum)	32	<2	<2	32
IFN-gamma (spont)	16	<2	16	<2
IFN-alpha (NDV)	256	256	<2	256
IFN-gamma (SEA)	64	<2	64	<2

IFN-alpha (NDV) preserved some activity (256 U/ml without treatment, 16 U/ml after treatment). IFN-gamma (spont) was completely inactivated following the exposure at 37 °C for 5 days. None of slight change in activity of IFN/AL (IV), IFN/AL (serum) and IFN-alpha (NDV) was observed following the exposure to 57 °C for 1 hr. Additional treatment with acid caused loss of activity of IFN/AL (IV) and IFN/AL (serum) titers below 1:2, whereas IFN-alpha (NDV) was only partially inactivated. IFN-gamma (spont) and IFN-gamma (SEA) completely lost their activity following exposure at 57 °C for 1 hr.

The comparative studies of these various IFN in terms of development of antiviral state in human diploid fibroblast culture showed that IFN/AL (IV), IFN/AL (serum), IFN-gamma (spont) and IFN-gamma (SEA) induced the 30 % antiviral state within 5–5.5 hr, whereas for IFN-alpha (NDV) it took 3.5 hr.

Table 3. Properties of various types of IFN

IFN type	IFN titer (U/ml)						
	Initial	37 °C/5 days		Initial	57 °C/1 hr		30 %*
		-	pH 2.0		-	pH 2.0	
IFN/AL (IV)	64	64	<2	8	8	<2	5.0
IFN/AL (serum)	32	32	<2	32	16	<2	5.5
IFN-gamma (spont)	16	<2	<2	8	<2	<2	5.5
IFN-alpha (NDV)	512	256	16	128	128	16	3.5
IFN-gamma (SEA)	128	128	2	16	<2	<2	5.5

* The time with the 30 % antiviral state develops in the human diploid fibroblast culture

Discussion

AID are usually characterized by inhibited cell-mediated immunity and increased polyclonal activation of B-cells. Therefore it seems important to elucidate the role of endogenous cytokines including IFN in these disorders. IFN/AL (serum) has been detected in the blood of patients with systemic lupus erythematosus (SLE), RA and AIDS, which are classified as AID by a number of investigators (Skurkovich and Eremkina, 1975; Hooks *et al.*, 1979; Preble *et al.*, 1982; Green and Spruance, 1984). The possible source of this IFN production was seen in the lymph node lymphocytes (Preble *et al.*, 1982), B-lymphocytes of blood (Capobianchi *et al.*, 1988), and cells in local inflammatory foci (Minagawa *et al.*, 1989). IFN/AL (serum) was also detected in RA and SDS patients' blood samples in our studies. However, blood leukocytes cannot be the source of IFN/AL as they seem produce to spontaneously IFN gamma *in vitro*. The latter was not detected in the blood apparently owing to its significant thermolability, which is more marked than that of the induced IFN-gamma (SEA).

IFN-gamma (spont) production by RA and SDS patients' leukocytes is probably one of the links involved in the pathogenesis of these two diseases. It cannot be excluded (Falcoff, 1972) that antilymphocytic globulins circulating in the blood in AID (Ljampert, 1988) could induce IFN-gamma in leukocytes. It is of interest that IFN-gamma promotes the production of antilymphocytic immunoglobulin (Golbus *et al.* 1988). However, it is still obscure what is the inducer and which cellular system is the producer of IFN/AL detected in the blood of AID patients. Shiozawa *et al.* (1988) reported higher levels of IFN/AL-alpha in the blood of an AID patients that had concomotant vasculitis. We have demonstrated the capacity of vascular endothelial cells of human umbilical vein to produce virus-induced IFN/AL-alpha (Scheglovitova *et al.*, 1989).

Previous publications demonstrated reduced production of induced IFN-alpha and IFN-gamma by AID patients' leukocytes *in vitro* (Shiozawa *et al.*, 1988; Stolzenburg *et al.*, 1988) and we have developed the test for assessing the capacity donors' leukocytes to produce IFN/AL (IV) (Scheglovitova *et al.*, 1990). In our trial IFN/AL (IV) and IFN-gamma (SEA) production by these patients' leukocytes was lower as compared with donors, the difference being statistically significant. The IFN-alpha (NDV) and IFN/AS (IV) production by patients' leukocytes was in these tests at the level of that by donors. The decreased production of IFN-gamma (SEA) and of IFN/AL (IV) reflects impaired functioning of T- and B- lymphocytes of RA and SDS patients. It appears possible that reduced production of induced IFN-gamma (SEA) results from hyporeactivity of T-cells already producing IFN-gamma. In patients with AID, B-lymphocytes seem hyporeactive (Egeland *et al.*, 1982). Decreased production of IFN/AL-alpha (IV) may be accounted for by poor IFN production capacity of blast forms of B-cells. However, it cannot be ruled out that the continuous presence of IFN in patients' blood leads to a hyporeactivity arising in both cellular populations.

Induced IFN/AL-alpha (IV), IFN/AL (serum) and IFN-gamma (spont) resemble IFN-gamma (SEA) by their sensitivity to pH 2.0 and time course of the antiviral state development in monolayer cell culture.

References

Archer, D. L., Smith, B. G., Ulrich, J. T., and Johnson, H. M. (1979): Immune interferon induction by T-cell mitogens involves different T-cell subpopulations. *Cell Immunol.* **48**, 420–426.

Capobianchi, M. R., De Marco, F., Di Marco, P., and Dianzani, F. (1988): Acid-labile interferon alpha production by peripheral blood mononuclear cells stimulated by HIV-infected cells. *Arch. Virol.* **99**, 9–10.

Egeland, T., Lea, T., Saari, G., Mellbye, O., and Natvig, J. (1982): Quantification of cells secreting rheumatoid factors of IgG, IgA and IgM class after elution from rheumatoid synovial tissue. *Arch. Rheum.* **25**, 1445–1449.

Falcoff, R. (1972): Some properties of virus and immune-induced human lymphocyte interferons. *J. gen. Virol.* **16**, 251–253.

Golbus, J., Salata, M., Greenwood, J., Hudson, J., and Richardson, B. C. (1988): Increased immunoglobulin response to α-interferon by lymphocytes from patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **46**, 129–140.

Green, J. A., and Spruance, S. L. (1984): Acid-labile alpha interferon. *New Engl. J. Med.* 922.

Hooks, J. J., Moutsopolos, H. M., Geis, S. A., Stahl, N. I., Decher, J. L., and Notkins, A. L. (1979): Immune interferon in the circulation of patients with autoimmune disease. *New Engl. J. Med.* 301, 5.

Ljampert, I. M. (1988): Autoantibodies reacting with skin basal epithelium and thymus endocrine epithelium – the first stage of development of various autoimmune processes. *Immunology* **4**, 5–9.

Minagawa, T., Asano, M., Nakane, A., Numata, A., Kohanawa, M., Kaneko, F., Hiraga, Y., Hachimori, K., Negishi, M., Yamazaki, S., and Sato, Y. (1989): Detection of interferons in sera and exudates or extracts of local lesion obtained from patients with immunological diseases, pp. 363–368. In Y. Kawade and S. Kobayashi (Eds): *Local Organizing Committee of the Fifth Annual Meeting of the Interferon Research. The Biology of the Interferon System 1988.*

Preble, O. T., Black, R. J., and Friedman, R. M. (1982): Systemic lupus erythematosus: Presence in human serum of an unusual acid-labile leukocyte interferon. *Science* **216**, 429–431.

Preble, O. T., Rothko, K., Klippel, J. H. Friedman, R. M., and Johnston, M. I. (1983): Interferon-induced 2-5 adenylate synthetase *in vivo* and interferon production *in vitro* by lymphocytes from systemic lupus erythematosus patients with and without circulating interferon. *J. exp. Med.* **157**, 2140–2146.

Ricci, M., Prete, G., Rossi, O., and Romagnini, S. (1988): Organ-specific autoimmunity. Pathogenic aspects and effector mechanisms in experimental and clinical diseases. *Ital. J. Med.* **4**, 68–75.

Scheglovitova, O. N., Antonov, A. S., Kabaeva, N. V., and Orlova, T. G. (1989): Interferon production by the primary culture of human endothelial cells. *Bull. exp. Biol. Med.* **11**, 580–583.

Scheglovitova, O. N., Kulieva, A. M., Kognovitskaya, A. I., and Mamontova, T. V. (1989): Characterization of α-acid labile human interferon. *J. Interferon Res.* **9**, Suppl. 2, A4–28.

Scheglovitova, O. N., Kulieva, A. M., and Orlova, T. G. (1990): Peculiarities of α-interferon production by human leukocytes. *Acta virol.* **34**, 529–536.

Shiozawa, S., Shiozawa, K., Imura, S., Yoshikawa, N., Saigo, K., and Kita, M. (1988): A sensitive radioimmunoassay of circulating interferon in viral and autoimmune diseases: effective α-interferon therapy for rheumatoid arthritis adult T-cell leukemia in which plasma α-interferon is low, pp. 97–102. *Proc. of the Fifth Annual Meeting of the ISIR.* Kyoto, Japan.

Skurkovich, S., and Eremkina, E. (1975): The probable role of interferon in allergy. *Ann. Allergy* **35**, 356–360.

Stolzenburg, T., Binz, H., Fontana, A., Felder, M., and Wagenhalaser, F. (1988): Impaired mitogen-induced interferon-γ production in rheumatoid arthritis and related diseases. *Scand. J. Immunol.* **27**, 73–81.