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MONOCLONAL ANTIBODIES NEUTRALIZING HUMAN LEUKOCYTE ACID- AND THERMOLABILE INTERFERON ALPHA

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Summary. — Using a high titred human leukocyte IFNα preparation which contained both acid- and thermolabile (AL-IFNα) and acid- and thermostable IFNα species in 9 : 1 proportion for immunization of BALB/c mice, five hybridomas secreting monoclonal antibodies that reacted with AL-IFNα were obtained. In antiviral and antiproliferative tests on HL-60 cells, their products showed high degree of specificity for AL-IFNα. The results suggest that both the "normal" leukocyte AL-IFNα and the IFNα found in sera of autoimmune and other chronic patients might belong to the same subtype of IFNα.

Introduction

Production of interferon (IFN) by cells both *in vitro* and *in vivo* is, as a rule, a short lasting phenomenon (Stewart, 1979; Buchmeier *el al.*, 1980). The fast disappearance of IFN from circulation is in accordance with the concept that its prolonged presence might be incompatible with the normal functioning of organs (Bocci, 1987). However, in several chronic diseases such as autoimmune, AIDS, Down's syndrome etc, persistent or reappearing IFN titres could be demonstrated (Hooks *et al.*, 1981; Preble *et al.*, 1984; Funa *et al.*, 1984). The pathogenic role of such IFN is not clear. However, the so called TRI-inclusions in SLE or in chronic hepatitis B showed a correlation with the presence of IFN in sera of lupus (SLE) patients and/or the cycles of IFN therapy in hepatitis B (Rich *et al.*, 1986; Grimley *et al.*, 1983). Characterization of IFN in above mentioned pathological conditions is rather tedious, to a great deal due to acid- and thermolability of such IFNs and because of its usually low concentration in the serum of patients. In majority of cases, the IFN found in chronic diseases was classified as IFNα based on neutralization with polyclonal anti-IFNα serum, but in Behcet's syndrome — as IFNγ (Ohno *et al.*, 1982), and both α and γ IFNs were found in psoriasis and lupus — SLE by others (Livden *et al.*, 198; Hooks *el al.*, 1982).

Chadha *et al.* (1985) reported that, when assayed before acid processing, an acid- and thermolabile component can be found in "normal" leukocyte IFNα preparations. We have come to similar conclusion (Borecký *et al.*,

1986) and recently Mécs *et al.* (1989) confirmed the presence of thermolabile IFNα components by electrofocusing of commercial IFNα preparations.

In this paper we report on obtaining monoclonal antibodies which differentiate selectively the acid- and thermolabile IFNα (AL-IFNα) from the acid- and thermostable IFNα species.

Materials and Methods

Interferons. The acid- and thermolabile IFNα (AL-IFNα) was induced in human leukocytes (rid of erythrocytes with 0.83 % NH_4Cl) with 200 haemagglutinating units (per 10^6 leukocytes) of Newcastle disease virus, strain B1 (NDV) suspended in Eagle's basal medium. After incubation at 37 °C for 16 hrs, the bulk of inducer was removed by high speed centrifagtion (30000 rev/min for 60 min, Beckman, rotor 70.1). As judged from the results of retitration of antiviral actvity (AV) on human embryonic lung fibroblasts in 19th passage, about 10 % of the AV activity was thermostable in this preparation. Its total AV activity (against vesicular stomatitis virus — VSV) was 1.28×10^5 units per ml. Other IFN preparations used: The acid- and thermostable human leukocyte IFNα was product of Celltech, G. Britain, Batch No. 527507. It contained 10^5 AV units per ml. Another preparation of human loukocyto IFN was produced in Instituto of Virology, Bratislava. It contained 10^6 units of IFN per mg. The human fibroblast IFN (Toray Ind, Japan, Lot L-0631) contained 10^7 units of IFN per mg. The human leukocyte IFN (Finnish Red Cross, Helsinki, Lot G-8510) contained 10^6 units of IFN per ml. Recombinant interferons, rIFNα1 and rIFNα2 were products of Boehringer, FRG, through courtesy of Dr. G. Bodos Vienna. Their AV activities were 10^8 units per ml for IFNα1 (Lot No. 648/7-10), and 5×10^6 unit per ml for IFNα2 (Lot No. 30404). The recombinant IFNαN was a gift of Dr. E. J. Gren (Latvijan Academy of Sciences, Riga). It is a partially purified preparation containing 2×10^5 units of IFN per ml.

Interferon assay. The antiviral activity (AV) of IFN preparations was assayed by cytopathic effect reduction method on microplates (96 well plates, Koh-i-noor, Czechoslovakia) using human diploid lung cells and vesicular stomatitis virus (VSV) as challenge. The titres were calibrated against the N.I.H. (U.S.A.) reference standard preparations of IFNα, β, and γ (kindly provided by Dr. Laughlin).

Immunization. Eight-week-old female BALB/c mice (Institute of Virology, Bratislava) were immunized at 7 week intervals for 5 months. The preparation of acid- and thermolabile IFNα (AL-IFNα) with 1.28×10^5 units of IFN per ml was emulsified with complete Freund's adjuvant in 1 : 1 ratio and aliquots of 0.5 ml (3.2×10^4 units) per mouse were injected intraperitoneally. After receiving 3 doses of immunogene, the mice were boosted intravenosly with 4.3×10^4 units of IFN in 0.3 ml of Eagle s basal medium. Three days later, the mice werue bled, sera collected and the spleens used for preparation of hybridomas.

Preparation of hybridomas. The fusion procedure and selection of hybridomas was performed as described in our previous paper (Kontsek *et al.*, 1988). Briefly, 10^8 spleen cells were fused with 2×10^7 NSO myeloma cells using PEG 1550 (Serva, FRG). Afterwards thecells were resuspended in Dulbecco's minimal essential medium (Sigma, FRG) with hypo anthine-aminopterine-thymidine and 15 % of a horse serum selected for cultivation of hybrixomas (Elan-clone, Czechoslovakia) and seeded in 96-well plates (Flow Lab., G. Britain). Aftd incubation at 37 °C in 5 % CO_2 atmosphere for 14 days, the culture supernatants from wells erth growing hybridomasd were collected and used for primary screening. The clones producingwiantibodies were reclone using the limiting dilution method on aa feeder layer from spleencells of BALB/c mice until stable subclones producing monoclonal ntibodies (MoAb) to AL-IF N were obtained.

Neutralization of antiviral (AV) activity of IFN. The primary screening of hybridomas was based on ability of culture supernatants to neutralize the AV activity of AL-IFN. Aliquots (0.05 ml) of hybridoma culture supernatants (in several parallels) were mixed with an equal volume (0.05 ml) of three-fold dilutions of IFNs tested. The mixtures were added to human diploid lung fibroblasts in microplates- as in IFN assay. Hybridomas secreting MoAbs which neutralized at least 3 units of IFN were considered positive. In several tests, standard polyclonal anti-IFNα, anti-IFNβ and anti-IFNγ sera, obtained through courtesy of Dr. Laughlin (N.I.H Bethesda, U.S.A.), were also employed. Monoclonal antibodies against conventional IFN α, β, γ used in this study were prepared in this Institute (Novák *et al.*, 1986; Kontsek *et al.*, 1988, 1989).

Neutralization of antiproliferative (AP) activity of IFN. The ability of hybridoma prod (MoAb) to neutralize the AP activity of IFN was assayed on HL-60 cells according to Kon *et al.* (1988). Briefly, into the microculture wells containing the mixture of 0.05 ml of tse (100 units per ml) and 0.05 ml hybridoma culture supernatant, 2×10^4 HL-60 cells in 0.1 m were added. After 72 hrs the number of cells were counted using a Bürker-chamber. As co the culture supernatant from Ig nonproducing cells was used. Each culture supernatant wa tested in quadruplicate and the results are expressed as mean of their number in each well. Cells used for AP assay were the HL-60 human promyelocytic cell line cultivated in PMI-1640 (Serva, F.R.G.) medium in presence of 10 % horse serum.

Quantitation of monoclonal antibodies in the culture supernatants: The amc unts of antibodies (Ig) in hybridoma culture supernatants were assayed by the sandwich method of ELISA. The wells in microculture plates Koh-i-noor, Czechoslovakia) were coated with swine anti-mouse immunoglobulin (SEVAC), Czechoslovakia). The detectin g antibody was a peroxidase-coniugated antimouse antibody (SEVAC, Czechoslovakia).

Determination of immunoglobulin types of monoclonal antibodies. The immunoglobulin type (Ig) of MoAbs was determined by ELISA using swine anti-mouse Ig and anti-isotype sera against mouse Ig conjugated with horse-radish peroxidase (Serotec, FRG).

Preparation and purification of monoclonal antibodies. 5×10^6 hybridoma cells (clone T-18) were inoculated into 4 weeks old BALB/c mice intraperitoneally. After 14 days, the ascitic fluid of mice was collected, clarified by centrifugation (20 000 rev/min for 30 min) and the Ig (in clone T-18) was precipitated with ammonium sulphate at 50 % saturation. The globulin was dissolved in distilled water and dialyzed against buffered saline at pH 7.2. The Ig concentration in the final product was determined by Lowry s method using bovine serum albumin as standard.

Results

When BALB/c mice were immunized with a high titred AL-IFNγ preparation, five hybridomas secreting Ig that neutralized the AV activity of AL-IFNα were obtained. Three of them were of IgG type and two of IgM type. The highest concentration of Ig (by ELISA) determined was produced by hybridoma T-18 (antibody activity 1 : 1024) and this MoAb was used

Table 1. Characterization of monoclonal antibodies neutralizing AL-IFN

Hybridoma culture supernatant	Ig type	Ig-concentration[a] (ELISA-titre)	Neutralization of IFN-activity: AV[b] units/ml	AP[c] effect of 50 units/ml
T1	G1	1 : 256	24	88 %
T4	G2a	1 : 512	48	100 %
T18	M	1 : 1024	48	100 %
T19	M	1 : 256	12	76 %
T24	G1	1 : 64	3	38 %
(NSO supern.[d])	0	0	0	0 %

[a] Concentration of Ig in culture supernatants determined by ELISA.

[b] Neutralization titre of the AV activity (in units per ml) after mixing 0.05 ml of AL-IFNα with 0.05 ml of hybridoma supernatant.

[c] Neutralization of AP activity of 50 units per ml of IFN in 0.05 ml after mixing with 0.05 ml of hybridoma supernatants (100 % neutralization corresponds to the number of cells incubated in absence of IFN, while 0 % neutralization corresponds to the number of cells found after incubation with IFN).

[d] Control — culture supernatantg-nonsecremyeloma cells NSO.

Table 2. Neutralization of antiproliferative effect of human natural and recombinant alpha interferons with anti-AL-IFNα antibodies T1, T18, and T19

Anti-AL-IFNα antibodies	AL-IFNα (4×10^2 U per ml)[p]		IFNα1[a] (4×10^2 U per ml)		IFNα[b] (5×10^2 U per ml)		IFNα[c] (1×10^2 U per ml)		Control (Without IFN)	
T-1[d]	99[g]	(99 %)	71	(71 %)	71	(71 %)	59	(59 %	100	100 %
T-18[g]	99	(98 %)	72	(71 %)	77	(76 %)	57	(56 %	)101	(100 %
T-19[g]	100	(98 %)	75	(73 %)	77	(75 %	56	(55 %)	102	(100 %)
No antibody	71	(69 %)	73	(71 %)	75	(73 %)	57	(55 %)	102	(100 %)

[a] Human recombinant IFNα 1 (Boehringer, Wien).
[b] Human leukocyte IFNα (Institute of Virology, Bratislava).
[c] Human leukocyte IFNα (Celltech, G. Britain).
[d] Anti-AL-IFNα monoclonal antibody — supernatant preparation.
[g] Anti-AL-IFNα monoclonal antibody — purified ascitic preparation.
[p] Number of IFN units added to cells.
[g] Number of cells $\times$ 10^4/ml after incubation with IFN and antibody for 72 hrs.

subsequently in most tests. The antiviral and antiproliferative activity of hybridoma Ig products showed a clear correlation with their Ig content (Table 1). The anti-AL-IFNγ monoclonal antibodies effectively neutralized the AP activity of AL-IFNα but were ineffective in neutralization of AP activity of thermostable IFN types IFNα, products of Celltech and/or Institute of Virology, Bratislava, or subtype α 1 (product of Boehringer, FRG) (Table 2).

When the concentrated T-18 monoclonal antibody against AL-IFNα was tested against the natural leukocyte IFNα (Celltech), natural fibroblast interferon β (Toray Ind.), natural (leukocyte) interferon γ (Helsinki) and recombinant IFN subtypes α1, α2, and αN — a high specificity of this monoclonal antibody for its inducer AL-IFN could be demonstrated (Table 3).

Table 3. Neutralization of the anti-proliferative activity of human IFNs with monoclonal antibody T-18 (anti-AL-IFNα) in HL-60 cells

IFN species (100 units per ml)	Neutralizing capacity[a] of monoclonal antibody T-18
AL-IFNα	0.15 μg per ml
S-IFNα (Celltech)	> 10μg per ml
rIFNα1 (Boehringer)	> 10 μg per ml
rIFNα2 (Boehringer)	> 10 μg per ml
rIFNαN (Riga)	> 10 μg per ml
IFNβ (Toray)	> 10 μg per ml
IFNγ[b] (Helsinki)	> 10 μg per ml

[a] Amount of MoAb "T-18" which neutralized 50 per cent of AP activity of tested IFN species.
[b] 10 units per ml of IFNγ were used in test.

Table 4. Neutralization antiproliferative effects of various human interferon types by monoclonal antibodies

Monoclonal antibody	AL-IFNα 200 U per ml	rIFN-α 2 100U per ml	IFN-β 100 U per ml	IFN-γ 10 U per ml	Control (without IFN)
Anti-AL-IFNα "T-18"[a]	65.3[c] (105 %)	49.3 (79 %)	50.7 (81 %)	51.2 (81 %)	62.4 (100 %)
Anti-AL-IFNα "T-18"[b]	70.9 (99 %)	ND	ND	ND	71.8 (100 %)
Anti-IFNβ "13"[a]	55.9 (80 %)	53.2 (76 %)	66.0 (95 %)	ND	69.5 (100 %)
Anti-IFNγ "A8"[a]	49.7 (80 %)	ND	ND	65.0 (105 %)	62.0 (100 %)

ND: not done.
[a] Ascitic fluid 1 : 100.
[b] Cell supernate from 1.5×10^6 hybridomas.
[c] Number of cells $\times$ 10^4/ml after incubation with IFN and antibody for 72 hrs.

While the AL-IFNα was neutralized by 0.15 μg per ml of T-18 monoclonal antibody, amounts exceeding 10 μg per ml T-18 antibody were ineffective in neutralization of other α, β, and γIFNs.

The selective neutralizing capacity of anti-AL-IFNα monoclonal antibody has been confirmed in tests where the T-18 monoclonal antibodies were compared with the neutralizing capacity of anti-IFNβ and anti-IFNγ monoclonal antibodies (Table 4). The high selectivity of anti-AL-IFNα antibodies seems to include also the "pathological" IFN species such as found in SLE and/or psoriatic patients. This is shown in Table 5 and suggests that the AL-IFNα found in "normal" leukocyte IFNα preparations which were induced *in vitro*, and, the "pathological" acid- and thermolabile IFN might be antigenically similar if not identical.

Table 5. Antiviral activity of AL-IFNα and its neutralization by polyclonal and monoclonal antibodies

Interferon species	pH-2 stability	Polyclonal[1] against IFN			Monoclonal against IFN			
		α	β	γ	α(stable)[2] (60/4)	α(labile)[3] (T-18)	β[2] (B6)	γ[3] (A8)
Hu Leu IFN	+	27/3[4] (+)	27/27 (−)	27/27 (−)	27/3 (+)	27/9 (−)	27/9 (−)	27/0 (−)
Hu Leu IFN	−	81/9 (+)	81/81 (−)	81/27 (−)	81/27 (−)	81/9 (+)	81/27 (−)	81/27 (−)
SLE-IFN	−	32/8 (+)	32/32 (−)	32/32 (−)	ND	32/8 (+)	ND	ND
Psoriatic IFN	−	32/8 (+)	ND	32/16 (−)	ND	32/8 (+)	ND	ND

[1] NIH (U.S.A.) reference sera, 1 : 100.
[2] Monoclonal sera, ascitic fluid, 1 : 100.
[3] Concentrated antibodies (Ig), 1 μg per ml.
[4] Titre of IFN before: titre of IFN after incubation with antiserum.
+: 50μl of antibody dilution neutralizes more than 4 units of IFN per 50 μl.
−: The neutralizing ability is under 4 units of IFN per 50 μl.

Table 6. Neutralization of antiproliferative activity of various interferons in HL-60 cells with various anti-IFN antibodies

IFN species (100 units per ml)	Neutralization[a] by: Monoclonal antibodies to (μg per ml) AL-IFNα (T 18)	IFNα (60/4)	IFNβ (B6)	IFNγ (A8)	Polyclonal antibodies to (dilutions) AL-IFNα	IFNα (NIH)	IFNβ (NIH)	IFNγ (NIH)
AL-IFNα	0.15	>10	>10	>10	10^{-4}	<4	10^{-1}	5×10
IFNα[b]	>10	0.2	>10	>10	10^{-3}	10^{-4}	10^{-1}	10^{-1}
IFNβ	>10	>10	0.3	>10	10^{-1}	10^{-1}	10^{-4}	10^{-1}
IFNγ[c]	>10	>10	>10	0.1	10^{-1}	10^{-1}	10^{-1}	10^{-4}

[a] Amount (of Ig), dilution (of sera) which neutralized 50 per cent of AP activity of tested IFN.
[b] Human leukocyte interferon α (Institute of Virology, Bratislava).
[c] 10 units per ml of IFNγ were used in test.

Comparison of the specificity of monoclonal and polyclonal anti-IFN sera revealed the presence of anti-IFNα antibodies in polyclonal anti-AL-IFMα serum and, of anti-AL-IFN antibodies in polyclonal anti-IFNγ serum (Table 6). This finding sheds some light on results of neutralization tests with SLE and other interferons with polyclonal antibodies as reported in the literature.

Discussion

By analogy with IFNγ, existence of an acid- and thermolabile IFNα subspecies (subtype?) which would be antigenically distínct from the known IFNα subtypes — cannot be *a priori* excluded. The candidates for such an IFNγ subtype are the IFNs found in autoimmune and several other chronic diseases such as AIDS or Down's syndrome (Hooks *et al.*, 1982; Waschke and Diezel, 1984; Preble *et al.*, 1984). However, a final antigenic classification of these IFNs meets difficulties. While the majority of authors found such IFNs to be of α type, others (Hooks *et al.*, 1982; Livden *et al.*, 1985) considered the results of neutralization tests supporting the view that several antigenic types of IFN may be present in sera of autoimmune patients. They might be produced by a hyperactive leukocyte(?) cell system resembling the enhanced activity of B-cell system in autoimmune diseases. However, the low concentrations of IFN found in sera of patients, its intermittent presence (Borecký *et al.*, 1986) as well as acid- and thermolability presents technical problems by performing identification tests and makes their results uncertain. The uncertainty extends also to polyclonal anti-IFN sera employed in such tests. Elsewhere (Borecký *et al.*, in press) we showed that polyclonal anti-IFN (anti-IFNα and anti-IFNγ) sera may contain, in various proportions, antibodies that are specific for AL-IFNα. This IFNα species is, however, often removed from IFN preparations during processing. An acid- and thermolabile preparation of IFNα containing the AL-IFNα component in 9 : 1 pro-

portion to the acid- and thermostable IFNα was used for obtaining monoclonal antibodies against the AL-IFNα. Surprisingly, several hybridoma products showed high neutralizing specificity for AL-IFNα when tested in antiviral and/or in antiproliferative tests. The antigenic distinctiveness of AL-IFNα found support also in tests where the neutralizing capacity of anti-AL-IFNα monoclonal antibodies (Table 3 and 4) were tested against rIFNα 1, rIFNα 2, and/or rIFNα N. However, obviously more subtypes should be tested for crossreactivity with anti-AL-IFNα sera before the final conclusion is met.

Several conclusions can be drawn from this study. First, they suggest that the AL-IFNα might represent an antigenically differing new subspecies of IFNα family. Second, such subspecies (as a developmental form?) might be present both in „normal" leukocyte IFNα preparations and in sera of patients with chronic autoimmune and/or other discases. Third, the AL-IFNα components may disappear from IFN preparations during their purification, sterilizatíon, storage etc. Fourth, if present in an IFN preparation and used for immunization, AL-IFNα may, in addition to the major IFN component, elicit production of specific antibodies which may react with AL-IFNα in tested IFN preparations and lead to faise conclusions. Fifth, as follows from correlation found between interferonaemia and appearance of damaged blood cells, in autoimmune disease, AIDS etc, the AL-IFNα may participate on the pathogenesis of several chronic diseases (TRI-inclusions, chromosome- and membrane damage etc, Rich *et al.*, 1986 etc). Sixth, although the AL-IFNα from "normal" leukocyte IFN preparations and the IFNs appearing during pathological conditions (SLE, psoriasís) seem to be similar, further comparative studies and involvement of further IFNα subtypes in cross-reactivity tests seem to be warranted.

References

Bocci, V. (1937): Metabolism of protein anticancer agents. *Pharmac. Ther.* **34**, 1—49.

Borecký, L., Kontsek, P., Novák, M., and Lackovič, V. (1989): Antigenic properties of human acid- and thermolabile interferon alpha (in preparation).

Borecký, L., Lackovič, V., Marcinka, K., and Rovenský, J. (1986): The interferon system in SLE and psoriatic patients. In B. Filipič (Ed.): *Yougoslav Colloqium on Interferon*, Sloven. Microb. Soc. Ljubljana, 73—79.

Buchmeier, M. J., Welsh, R. M., Dutko, F. J., and Oldstone, M. B. A. (1980): The virology and immunology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* **30**, 275—326.

Funa, K., Auneren, G., Alm, G. V., and Björksten, B. (1984): Abnormal interferon production and NK cell responses to interferon in children with Down's syndrome. *Clin. exp. Immunol.* **56/3**, 493—500.

Chadcha, K. C. (1985): Acid labile human leukocyte interferon. In F. Dianzani and G. B. Rossi (Eds): *The interferon System*, Raven Press, New York, 35—41.

Grimley, P. M., Kanz, Y. H., Silverman, R. H., Davis, G., and Hoofnagle, J. H. (1983): Blood lymphocyte inclusions associated with alpha interferon. *Lab. Invest.* **48**, 30A.

Hooks, J. J., Jordan, G. W., and Cupps, T. (1982): Multiple interferons with the circulation of patients with systemic lupus erythematosus and vasculitis. *Arthritis Rheum.* **25**, 390—400.

Hooks, J, J., Moutsoupoulos, H. M., and Notkins, A. L. (1981): Circulating interferon in human autoimmune diseases. *Tex. Rep. Biol, MeW.* **41**, 164—168.

Kontsek, P., Borecký, L., Kontseková, E. and Novák, M. (1989): Murine monoclonal antibodies against human fibroblast (beta) interferon. Correlation of antiviral and antiproliferative activity. *Acta virol.*, in press.

Kontsek, P., Borecký, L., Kontseková, E., Šujanová, Z., and Novák, M. (1988): Monoclonal antibodies neutralizing the antiviral and the antiproliferative activities of human interferon gamma. *Acta virol.* **32**, 334—338.

Livden, J. K., Bjerke, J. R., Degré, M., and Matre, R. (1986): The effect of Goeckerman therapy on interferon in serum and suction blister fluid from patients with psoriasis. *Brit. J. Dermatol.* **114**, 217—225.

Mécs, I., Tóth, S., and Sirokmán, F. (1988): Effect of storage and ^{60}Co irradiation on the antiviral activity and subtype pattern of native human interferon-alpha preparation. *Acta virol.* **33**, 177—182.

Novák, M., Borecký, L., Kontseková, E., Kontsek, P. and Nováková, M. (1986): Preparation and characterization of hybridomas producing monoclonal antibodies against human alpha interferon. *Acta virol.* **30**, 228—233.

Ohno, S., Kato, F., and Matsuda, H. (1982): Detection of gamma interferon in the sera of patients with Behcet's disease. *Infect. Immun.* **36**, 202—208.

Preble, O. T., Hook, A. H., Quinnan, G. V. (1984): Role of interferon in AIDS. *Ann. N. York Acad. Sci.* **437**, 65—75.

Rich, S. A., Owens, T. R., Auzola, M. C., and Bartholomew, L. E. (1986): Induction of lupus inclusions by sera from patients with systemic lupus erythematosus. *Arthritis Rheum.* **29**, 501—507.

Stewart, W. E. II. (1979): *The Interferon System.* Springer Verlag, Wien—New York.

Waschke, S., and Diezel, W. (1984): Interferon in patients with psoriasis. In L. Borecký and V. Lackovič (Eds.): *Physiology and Pathology of Interferon System*, Contr. Oncol. Karger, Basel, v. 20, 298—303.