mon, New York, 1978); A. Guidotti, G. Toffano, E. Costa, *Nature (London)* **275**, 553 (1978); M. Baraldi, A. Guidotti, J. P. Schwartz, E. Costa, *Science* **205**, 821 (1979); A. Guidotti, M. Baraldi, E. Costa, *Pharmacology* **19**, 267 (1979); A. Guidotti, G. Toffano, M. Baraldi, J. P. Schwartz, E. Costa, in *GABA-Neurotransmitters*, P. Krogsgaard-Larsen, J. Scheel-Kruger, H. Kofod, Eds. (Munksgaard, Copenhagen, 1979), p. 406.
5. E. Costa, A. Guidotti, G. Toffano, *Br. J. Psychiatry* **133**, 239 (1978).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
7. D. V. Greenlee, P. C. Van Ness, R. W. Olsen, *Life Sci.* **22**, 1653 (1978); P. R. Andreews and G. A. R. Johnston, *Biochem. Pharmacol.* **28**, 2697 (1979).
8. E. G. S. Spokes, N. J. Garrett, L. L. Iversen, *J. Neurochem.* **33**, 773 (1979).
9. M. L. Zeneroli, E. Iuliano, G. Racagni, M. Baraldi, *J. Neurochem.* **38**, 1219 (1982).
10. J. L. Waddington and J. Cross, *Nature (London)* **276**, 618 (1978); K. Kuriyama, E. Kurihara, Y. Ito, Y. Yoneda, *J. Neurochem.* **35**, 343 (1980).
11. A. Guidotti, K. Gale, A. Suria, G. Toffano, *Brain Res.* **172**, 566 (1979).
12. R. E. Rosenthal, *Anal. Biochem.* **20**, 525 (1967).
13. H. A. Feldman, *ibid.* **48**, 317 (1972).
14. This work was supported in part by Consiglio Nazionale delle Ricerche grant No. 289/79.019996.04/115.1158 and by an Italian Ministero Pubblica Instruzione grant (1979–1980).

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Systemic Lupus Erythematosus: Presence in Human Serum of an Unusual Acid-Labile Leukocyte Interferon

Abstract. *A previously undescribed species of human leukocyte, or alpha, interferon is present in the serum of many patients with systemic lupus erythematosus. It was shown to be α-interferon by neutralization with specific antiserums, affinity column chromatography, and antiviral activity on bovine cells. However, 23 of 30 interferon samples tested were inactivated by incubation at* p*H 2, a characteristic of human "immune," or gamma, interferon. Multiple samples of interferon from the same patient had similar biological properties, but samples from different patients were not all identical, suggesting that several variants of this species of human α-interferon may exist.*

Human interferons (IFN's) are classified into three groups on the basis of their antigenic properties (*1*). α-Interferon is produced mainly by leukocytes in response to a variety of viral and nonviral stimuli and is stable at *p*H 2. At least 12 distinct species of human α-IFN have been cloned (*2*), and some differ in biological properties (*3*). β-Interferon is synthesized predominantly by fibroblast-like cells and, to a much lesser extent, by leukocytes, and is also acid-stable. Gamma, or "immune," IFN is released by lymphocytes following exposure to mitogens or specific antigens (*4*). γ-Interferon is inactivated by incubation at *p*H 2 and is generally more heat-labile than α- or β-IFN (*4*, *5*). Both α- and γ-IFN may be involved in the regulation of immune responses in vivo.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by a disfiguring skin rash, arthritis, and nephritis. Individuals with active SLE may have high concentrations of circulating immune complexes, antibodies to single- or double-stranded DNA, or antibodies to several small nuclear or cytoplasmic ribonucleoprotein complexes (*6*). Many patients with SLE also have high concentrations of circulating IFN (*7*, *8*), which may contribute to the pathogenesis of the disease. Hooks *et al.* (*8*) concluded that γ-IFN was present in these patients because the IFN was inactivated at *p*H 2, and they hypothesized that circulating autoantibodies or immune complexes stimulated production of γ-IFN by sensitized lymphocytes.

The recent demonstration of distinct classes of α-IFN (*3*) prompted us to reexamine the type of IFN in patients with SLE by using more specific criteria to identify the IFN. Our data show that the IFN present in SLE is of the alpha type. This was determined by neutralization with specific antiserums, affinity column chromatography, and antiviral activity on nonhuman cells. However, unlike reference human leukocyte IFN, the α-IFN found in most IFN-positive SLE patients was inactivated by incubation at *p*H 2 and therefore appears to be a previously undescribed form of human α-IFN.

Serum samples from 138 SLE patients (*9*) were analyzed; 65 patients (47.1 percent) yielded one or more samples that contained significant (8 to 128 I.U./ml) concentrations of IFN. About 60 percent of these samples had IFN titers between 16 and 64 I.U./ml. In contrast, IFN was undetectable (≤ 4 I.U./ml) in serum samples from 22 control (healthy) individuals and from 13 patients with drug-induced SLE (*9*). Of 86 samples obtained during exacerbations of the disease, 45 contained IFN (8 to 128 I.U./ml). Only 25 percent of the samples obtained from patients during disease remission contained IFN. These values are similar to those obtained previously (*7*, *8*).

Standard human leukocyte IFN had equal antiviral activity on human and bovine cells (Table 1). Seventy-nine serum samples from 65 IFN-positive pa-

Table 1. Comparison of IFN from SLE patients with standard human α-, β-, and γ-IFN. Interferon in samples from SLE patients was quantitated by a semimicro method on human (GM 2504) or bovine (MDBK) cell cultures. About 2×10^4 cells were incubated (18 hours at 37°C) with twofold serial dilutions of IFN in 96-well microtiter plates. Virus was then added and incubation was continued for 24 to 36 hours. In experiment 1, vesicular stomatitis virus was used as the challenge virus for all assays. In experiment 2, all assays were performed on GM 2504 cells, and the challenge virus was encephalomyocarditis virus. Virus-induced cytopathic effects were evaluated microscopically, and the IFN titer was defined as the reciprocal of the highest dilution of sample to protect 50 percent of the cells. Standard α-, β-, and γ-IFN were included in each assay. Results were standardized to 023-901-527 reference human leukocyte IFN (National Institutes of Health). In experiment 2, 50 μl of IFN-positive SLE serum (16 to 100 I.U./ml) was mixed with 50 μl of medium or with 50 μl of antibodies to α-, β-, or γ-IFN (*10*). After incubation at 37°C for 60 minutes, residual IFN was assayed. Dilutions of antibody to IFN capable of specifically neutralizing 100 I.U. of homologous IFN per milliliter were used (dilution of antibody to α-IFN, 1:750; antibody to β-IFN, 1:150; γ-IFN, 1:10). The neutralization factor is the IFN titer of the medium controls divided by the titer of IFN remaining after treatment with antibody to IFN. Std, standard; N.T., not tested.

Experiment	Interferon	Number of samples	Titer on human cells (I.U./ml)	Titer on human cells/titer on bovine cells	Neutralization factor		
					Antibody to α-IFN	Antibody to β-IFN	Antibody to γ-IFN
1	Stdα	2	100	1 to 2			
	Stdβ	1	100	64			
	Stdγ	1	100	>32			
	SLE	64	8 to 128	0.5 to 2			
	SLE	15	8 to 128	4 to 8			
2	Stdα	2	100		30 to 40	0	0
	Stdβ	1	100		0	10 to 20	0
	Stdγ	1	100		0 to 2	0	30 to 60
	SLE	21	16 to 128		8 to 128	0 to 2	N.T.
	SLE	6	16 to 128		8 to 16	0 to 2	0
	SLE	5	10 to 100		2 to 4	0	0

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tients were assayed in duplicate on human and bovine cells. For 64 of the samples, the IFN titer on bovine cells was the same as the titer on human cells (within the twofold error inherent in the assay). Although the activity of the remaining 15 samples was consistently lower on bovine cells than on human cells, they were much more like α-IFN than like β- or γ-IFN.

Thirty-two of the 79 IFN samples were also analyzed (Table 1) with monospecific rabbit antiserum to human α-, β-, or γ-IFN (*10*). None of the samples tested was significantly affected by treatment with antibodies to β- or γ-IFN. However, the IFN in 27 of the 32 samples was significantly neutralized (8- to 128-fold) by antibodies to α-IFN and was therefore α-IFN by definition (*1*). Although 80 to 90 percent of the IFN in each sample was neutralized, residual antiviral activity was detectable in some of the serum samples, indicating only partial neutralization by the antiserum to α-IFN. The IFN in the remaining five samples (from two SLE patients) was neutralized only 50 to 75 percent. Since the IFN in these samples was not affected by antibodies to β- or γ-IFN, we concluded that it was also α-IFN. The antiserum to α-IFN was prepared against partially purified IFN produced by virus-stimulated human leukocytes. This IFN is a natural mixture of several subspecies of human α-IFN. Our neutralization data are consistent with the hypothesis that the predominant IFN in the circulation of SLE patients may be a minor component of the α-IFN produced in vitro. This theory is also supported by the finding that monoclonal NK-2 antibody to α-IFN (*11*) was more active against IFN from three different SLE patients (tenfold neutralization) than it was against standard virus-induced α-IFN (threefold neutralization).

Table 2. Affinity chromatography of SLE plasma on Sepharose with antibody to α-IFN. Plasma from one SLE patient (patient Ter in Table 3) was passed through a column containing rabbit antibody to α-IFN bound to Sepharose (*11*). The column was washed with phosphate-buffered saline and with 0.5*M* NaCl and then eluted with 0.5*M* NaCl containing 0.1*M* acetic acid (*p*H 2.5). One-milliliter fractions were collected, neutralized, and assayed for IFN as described in the legend to Table 1.

Fraction	Volume (ml)	Total IFN units	Percent recovery
Plasma	50	6400	100
Flow-through	50	<400	
PBS wash	5	<20	
0.5*M* NaCl wash	5	<20	
0.5*M* NaCl + 0.1*M* acetic acid	1	8	
	1	1024	16
	1	768	12
	1	384	6
	1	64	1
	1	24	0.3
	1	12	

Table 3. Properties of α-IFN in serum from eight SLE patients. IFN in serum samples from several of the SLE patients included in Table 1 were incubated at *p*H 2 (for 24 hours at 4°C) or for 60 minutes at 56°C. For incubation at *p*H 2, 100-μl portions of IFN-positive serum (16 to 100 I.U./ml) were slowly titrated to *p*H 2 by stepwise addition of 0.1*N* HCl (total volume, 6 to 10 μl, depending on the sample). Control samples received an equivalent volume of sterile distilled water. After incubation, samples were returned to *p*H 7 by stepwise addition of 0.1*N* NaOH; control samples again received water. Any precipitate was removed by centrifugation. The residual IFN was assayed on GM 2504 cells. Standard α-, β-, and γ-IFN samples, diluted to 100 I.U./ml in human type AB serum (Flow), were acidified, incubated, and neutralized in an identical manner. All serum samples were drawn between March and September 1981. Data for neutralization with antibody to β-IFN (Table 1) has been omitted since all results were negative.

Source of IFN	Sample	Titer (U/ml)		Neutralization factor		Inactivation factor	
		On GM 2504	On MDBK	Antibody to α-IFN	Antibody to γ-IFN	*p*H 2	56°C
Patient							
Bro	1	512	512	2	0	8	2 to 4
	2	512	512	2	0	0	0
	3	256	128	4	0	4	0
	4	256	512	4	0	4	0
Val	1	256	128	8 to 16	0	4	4
	2	512	512	8	N.T.	2	4
	3	256	128	4 to 8	N.T.	2	8
Rou	1	64	64	8	0	4	0
	2	128	32	>16	0	4	0
Lew	1	256	128	16	0	>8	0
	2	128	64	8	N.T.	>4	2
Waf		64	128	>4	0	8	N.T.
Ter		128	64	>16	N.T.	4	4
Fra		256	64	8	N.T.	>16	2
Bat		64	32	2	0	>2	4
Standard							
α		512	512	30 to 40	0	0	0
β		1000	16	0	0	0	0
γ		128	<4	0 to 2	30 to 60	16 to 32	>16

Table 2 shows the affinity column chromatography (*12*) results obtained with plasma from one IFN-positive SLE patient. The IFN was specifically bound to a column containing antibodies to α-IFN, but only 35 percent of the IFN activity was recovered after elution at *p*H 2.5. The IFN that was eluted from the column was completely neutralized with antiserum to α-IFN. These and previous results (*7*, *8*) led us to evaluate the acid sensitivity of the α-IFN found in our SLE patients. Interferon in 23 of 30 serum samples and three plasma samples was inactivated fourfold or more by incubation for 24 hours at *p*H 2 and 4°C. Reference human α- and β-IFN's were completely stable, and reference human γ-IFN was inactivated more than 16-fold under the same conditions. In addition, eight of the acid-labile α-IFN's from SLE patients were inactivated by heating at 56°C for 60 minutes. These results support the conclusion that the α-IFN in serum from SLE patients is a minor component of standard human leukocyte IFN. While serial samples from the same patient had similar biological properties, IFN samples from different patients were not all identical. Data on the α-IFN in the serum of eight representative SLE patients are given in Table 3.

Additional experiments showed that unstimulated purified mononuclear cells (separated on Ficoll-Hypaque gradients) from IFN-positive patients do not spontaneously secrete IFN in culture. Although others (*13*) recently investigated IFN production by leukocytes isolated from SLE patients, they neither mea-

sured serum IFN nor characterized the IFN made in vitro. We found that pokeweed mitogen (a B and T cell mitogen), but not phytohemagglutinin (a T cell mitogen), induced high levels (200 to > 2000 I.U./ml) of γ-IFN in mononuclear cell cultures from both IFN-negative patients and patients with α-IFN in their serum. Preliminary experiments suggest that SLE lymphocytes stimulated with Newcastle disease virus inactivated with ultraviolet radiation produce a conventional mixture of α-IFN's which is stable at *p*H 2. These results suggest that the acid-labile α-IFN in the serum of these patients may be synthesized by organ-bound lymphocytes or monocytes rather than by cells in the peripheral circulation. However, previous studies have also shown that the method of preparation of the cells before culturing, the nature of the accessory cells in the culture, and other factors may influence the type of IFN produced by leukocytes in vitro (*5*).

Our results strongly suggest that a particular type of α-IFN is specifically elevated in about 50 percent of all SLE patients. We do not know whether the acid-labile α-IFN found in SLE patients corresponds to any of the already known species of human leukocyte IFN or whether it is also found in patients with other diseases. Although IFN is more prevalent in patients with active SLE, we have not been able to correlate any individual serologic or clinical marker of disease with the presence of IFN in our group of patients. The presence of this form of IFN may be useful in the definition of subsets of SLE patients with hereditary or other factors in common which could contribute to development of the disease.

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References and Notes

1. Interferon Nomenclature Committee, *Nature (London)* **286**, 110 (1980).
2. S. Nagata, N. Mantei, C. Weissmann, *ibid.* **287**, 401 (1980); D. V. Goeddel *et al.*, *ibid.*, p. 411; M. Streuli, S. Nagata, C. Weissman, *Science* **209**, 1343 (1980); D. V. Goeddel, D. W. Leung, T. J. Dull, M. Gross, R. M. Lawn, R. McCandliss, P. H. Seeburg, A. Ullrich, E. Yelverton, P. W. Gray, *Nature (London)* **290**, 20 (1981); R. M. Lawn, J. Adelman, T. J. Dull, M. Gross, D. V. Goeddel, A. Ullrich, *Science* **212**, 1159 (1981).
3. E. Yelverton, D. Leung, P. Weck, P. W. Gray, D. V. Goeddel, *Nucleic Acids Res.* **9**, 731 (1981); P. Weck, S. Apperson, L. May, N. Stebbing, *J. Gen. Virol.* **57**, 233 (1981).
4. L. B. Epstein, *Tex. Rep. Biol. Med.* **35**, 42 (1978); in *Biology of the Lymphokines*, S. Cohen, E. Pick, J. Oppenheim, Eds. (Academic Press, New York, 1979), p. 443.
5. M. J. Valle, G. W. Jordan, S. Haahr, T. C. Merigan, *J. Immunol.* **115**, 230 (1975).
6. M. R. Lerner and J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5495 (1979); M. R. Lerner, J. A. Boyle, S. M. Mount, F. L. Wolin, J. A. Steitz, *Nature (London)* **283**, 220 (1980); M. R. Lerner, N. C. Andrews, G. Miller, J. A. Steitz, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 805 (1981).
7. S. Skurkovich and E. I. Eremkina, *Ann. Allergy* **35**, 356 (1975).
8. J. J. Hooks, H. M. Moutsopoulos, S. A. Geis, N. I. Stahl, J. L. Decker, A. L. Notkins, *N. Engl. J. Med.* **301**, 5 (1979).
9. Serum samples from 49 SLE patients and 19 healthy control individuals were provided by D. Koffler. Serum samples from 13 patients with drug-induced SLE and from three healthy controls were provided by E. Hess. The remaining samples were drawn from patients at the Clinical Center, National Institutes of Health.
10. Rabbit antibody to α-IFN was prepared by immunization with partially purified human leukocyte IFN (specific activity, > 10^6 U per milligram of protein). Serum samples obtained 106 days after the onset of immunization had a neutralization titer of 1:24,000 against standard α-IFN and 1:16 against standard β-IFN. The antiserum to rabbit β-IFN [described in J. Vilcek, S. Yamazaki, E. A. Havell, *Infect. Immun.* **18**, 863 (1977)] had a neutralization titer of 1:4000. Antibody to γ-IFN was prepared by immunization of rabbits with purified γ-IFN (a 20,000-dalton protein) isolated on sodium dodecyl sulfate–polyacrylamide gels [Y. K. Yip, B. Barrowclough, C. Urban, J. Vilcek, *Science* **215**, 411 (1981)]. The antiserum neutralizes γ-IFN components migrating at 20,000, 25,000, and 45,000 daltons. The titer of the antiserum to γ-IFN is about 1:400.
11. D. S. Secher and D. C. Burke, *Nature (London)* **285**, 446 (1980).
12. T. G. Hayes, Y. K. Yip, J. Vilcek, *Virology* **98**, 351 (1979).
13. P. A. Neighbour and A. I. Grayzel, *Clin. Exp. Immunol.* **45**, 376 (1981).
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Rediscovery of the Yellow-Fronted Gardener Bowerbird

Abstract. *The long-lost bowerbird* Amblyornis flavifrons *was found in the Foja Mountains of west New Guinea, and its bower and display were discovered. The bower is a stick tower on a rimmed moss platform, adorned with separate piles of fruit of three different colors. The displaying male extends toward the female a blue fruit set against his golden crest. These observations support a derivation of bower ornamentation from ritual courtship feeding and a transfer of ornamentation from the male's plumage to the bower.*

The most elaborate structures built by birds are the bowers of the family Ptilonorhynchidae (bowerbirds). These structures include walled avenues, huts 1 to 2 m in diameter, stick towers several meters high, carefully laid out lawns, and moss platforms with parapets. Bowers are decorated with colored objects such as fruits, flowers, shells, mushrooms, and (near human settlements) coins, marbles, bottle tops, and toothbrushes. At least four species orient their bower in a constant compass direction, and at least six use a tool to paint the bower with crushed plant matter, charcoal, or blue laundry powder (*1–4*).

Ever since 19th century explorers discovered bowers and mistook them for man-made structures, biologists have puzzled over the problems that bower building poses. These include the function and evolution of bowers, the roles of intelligence and aesthetic sense in their construction, the relation between bower ornamentation and male ornamental plumage, and the social system of the architects. Answering these questions has been hampered by the paucity of field observations of bowerbirds, in part because they are confined to New Guinea and Australia and often to remote areas there. Knowledge of the mating display at the bower is crucial for interpreting bower function. However, of the 15 species known or inferred to build bowers, the display is still unknown for seven, the bower itself for two.

Until the 1920's, vast numbers of skins of birds of paradise and bowerbirds were shipped from New Guinea by feather merchants. From these shipments, ornithologists described many taxa whose home grounds within the New Guinea region were kept secret by the merchants. By 1930, as a result of scientific collecting expeditions, these new forms had mostly been traced to their home grounds or shown to be hybrids, with a notable exception: the yellow-fronted gardener bowerbird *Amblyornis flavifrons*, a spectacular golden-crested bird known only from three adult male skins sold in 1895 to the Tring Museum by a plume merchant. The female remained unknown. A dozen expeditions searched remote areas of New Guinea for this species, without success (*2*).

In 1979 and 1981, I found *A. flavifrons* in the Foja (Gauttier) Mountains of west New Guinea, and discovered the bower,

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