INTERFERON α ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS IS NOT INTRINSICALLY ACID LABILE

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Interferons (IFN) are a class of inducible proteins that have been defined by their ability to protect cells from viral infections and are antigenically subclassified as IFN-α, -β, and -γ (1). IFN also possess many potent and diverse immunoregulatory activities (1). Elevated levels of IFN have been detected in the serum and plasma of many patients with systemic lupus erythematosus (SLE) and other autoimmune diseases (2-5), and also in patients with AIDS (6), a disorder now recognized to involve many SLE-like autoimmune phenomena (7). Notably, in SLE and in AIDS, serum IFN levels correlate positively with disease activity (2, 8). The IFN in these immunoregulatory disorders has been postulated to be one or more atypical subspecies of IFN-α that becomes inactivated after dialysis at pH 2, unlike other well-characterized IFN-α (3-6). Some investigators have used the term *autoimmune interferon* to describe this activity (9). Although it is an attractive possibility that structural differences in this IFN-α that cause acid lability may also constitute functional determinants that mediate the autoimmune effects seen in these diseases, there is no direct evidence for this assertion, and the true nature of acid lability is not known.

This study was conducted to examine the physicochemical properties of SLE-associated IFN-α (SLE IFN-α) to identify additional physical differences from known IFN and to eventually purify the molecule for further analysis. The results show that SLE IFN-α is physicochemically indistinguishable from conventional IFN-α and that acid lability is not an intrinsic property of SLE IFN-α, but rather the result of other factor(s) in SLE plasma.

Materials and Methods

Patient Population. Sera and plasma were collected from individuals who fulfilled at least four criteria for the diagnosis of SLE as established by the American Rheumatism Association (10). Normal sera and plasma were obtained from volunteer donors at the NYU Medical Center Blood Donor Program.

IFN Assays and Acid-Lability Tests. Antiviral activity was determined in duplicate by previously described methods (11). IFN titers are defined as the dilutions at which 50% of FS-4 fibroblasts are protected from the cytopathic effects of encephalomyocarditis virus 24 h after

infection and are expressed as IU/ml based on human IFN-α standard G-023-091-527 (National Institutes of Health, Bethesda, MD). Samples were tested for acid lability by dialysis in 100 mM glycine-HCl (pH 2) for 24 h at 4°C, followed by dialysis in PBS (pH 7.4) for another 24 h at 4°C (3). Control preparations were dialyzed in PBS throughout the 48 h.

Standard Interferons and Neutralizing Antibodies. NK-2 IFN-α is a natural preparation of different IFN-α produced by human lymphoblastoid cells (Celltech Limited, Berkshire, UK). Purified human rIFN-α2 and rIFN-β_{ser17} were from Schering Corp. (Bloomfield, NJ) and Cetus Corp. (Emeryville, CA), respectively. Human IFN-γ was prepared according to the method of Yip et al. (11). Polyclonal sheep anti-IFN-α antibodies were obtained from Interferon Sciences, Inc. (New Brunswick, NJ). Polyclonal bovine anti-IFN-β antibodies were provided by Dr. Jan Vilček (NYU School of Medicine, New York, NY). Polyclonal rabbit anti-IFN-γ antibodies were prepared by published methods (12). Antibody neutralization assays, described elsewhere (3), were conducted using preimmune sheep, bovine, or rabbit sera as negative controls. rIFN-α2 was radioiodinated with ^{125}I using the Bolton-Hunter reagent (reference 13; New England Nuclear, Boston, MA).

Fast Protein Liquid Chromatography (FPLC).[1] All chromatographic methods were performed using the FPLC System (Pharmacia Fine Chemicals, Piscataway, NJ). Anion exchange chromatography was done by loading samples onto the Mono Q HR 10/10 column equilibrated in 20 mM Tris (pH 7.5) and eluting with a linear gradient of NaCl from 0 to 700 mM at a flow rate of 4.00 ml/min. Chromatofocusing on the Mono P HR 5/20 column and gel filtration on the Superose 12 HR 10/30 column are described in the figure legends. Elution profiles were monitored by UV absorbance at 280 nm.

Miscellaneous Protein Manipulations. Protein preparations were concentrated in dialysis tubing using Aquacide II (Calbiochem-Behring Corp., LaJolla, CA).

Results

Physicochemical Characterization of SLE IFN-α. 360 serum and plasma samples from 104 SLE patients were screened for the presence of IFN activity, and in agreement with earlier reports (2, 3), 44 individuals (42%) exhibited significant IFN activity (>8 IU/ml) on at least one occasion, while no normal serum or plasma ($n = 7$) exhibited any IFN activity. The IFN activity from two SLE patients, DS and DT, was found to conform to the previously reported acid-labile IFN-α. The IFNs from both patients are reduced to <20% of original activity by anti-IFN-α antibodies, but are unaffected by antibodies against IFN-β and IFN-γ (Table I). This incomplete neutralization has been observed by others (3, 4). Simultaneous incubation of the SLE IFN with all three panels of antibodies abrogates the IFN activity to the same degree as with anti-IFN-α antibodies alone (data not shown), thus ruling out the presence of trace amounts of IFN-β or IFN-γ in the plasma. In addition, the results in Table II show that IFN activity from DS and DT is acid labile; standard IFN-α and IFN-β remain active after acid dialysis, while IFN-γ, known to be acid labile, is inactivated.

The charge properties of SLE IFN-α from DS and DT were compared with those of reference NK-2 IFN-α by FPLC/Mono Q anion-exchange chromatography. Both were found to bind avidly to the anion exchange column at pH 7.5, requiring 250 to 300 mM NaCl to elute from the column (data not shown). The similarity in charge properties between SLE and standard IFN-α was further confirmed by chromatofocusing experiments (Fig. 1). SLE IFN was eluted from the Mono P column by Polybuffer around pH 5.1 (panel *A*), while the bulk of NK-2 IFN-α was eluted within a pH range of 4.8 to 5.5 (panel *B*). All known subspecies of IFN-α have isoelectric points between 5.0 and 7.0 (11).

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[1] *Abbreviation used in this paper:* FPLC, fast protein liquid chromatography.

TABLE I

Antibody Neutralization of Interferons

Sample	IFN titer with antibody:			
	Preimmune	Anti-IFN-α	Anti-IFN-β	Anti-IFN-γ
		IU/ml		
NK-2 IFN-α	256	<2 (<0.8%)*	256 (100%)	256 (100%)
IFN-β	4,096	4,096 (100%)	384 (9.4%)	4,096 (100%)
IFN-γ	2,048	2,048 (100%)	2,048 (100%)	<2 (<0.1%)
DS-SLE (whole plasma)	384	64 (16.7%)	384 (100%)	384 (100%)
DS-SLE (Post-Mono Q)‡	192	32 (16.7%)	192 (100%)	192 (100%)
DS-SLE (Post-Mono Q/Superose 12)‡	64	8 (12.5%)	64 (100%)	64 (100%)
DT-SLE (whole plasma)	64	4 (6.3%)	64 (100%)	64 (100%)
DT-SLE (Post-Mono Q)‡	24	<2 (<8.3%)	24 (100%)	24 (100%)

Representative results from multiple experiments (n = 3).
* Parentheses denote percent activity relative to control incubation with preimmune sera.
‡ IFN-positive fractions were pooled after fractionation and concentrated before assay.

FPLC/Mono Q chromatography results in a 90–95% recovery and an approximately sixfold purification of SLE IFN activity (data not shown). Gel filtration of Mono Q-enriched SLE IFN on the Superose 12 column revealed a single peak of IFN activity, eluting in fractions corresponding to ~19 kD, which is within the molecular mass range of known IFN-α subspecies (14) and coincident with the elution of ^{125}I-rIFN-α2 (Fig. 2). Unpurified plasma SLE IFN-α also elutes in fractions corresponding to 19 kD (data not shown). This result and the high recovery yield confirm that Mono Q-purified SLE IFN is representative of that in unfractionated plasma. Further, antibody neutralization assays verify that Mono Q-purified IFN from DS and DT is specifically inhibited by antibodies against IFN-α (Table I).

SLE IFN-α is Acid Stable. More significantly, however, when Mono Q-purified SLE IFN-α from DS and DT was dialyzed in acid, the IFN activity was completely

TABLE II

Acid Stability of Interferons

Sample	IFN titer with dialysis buffer of:	
	PBS (pH 7.4)	100 mM Glycine-HCl (pH 2.0)
		IU/ml
NK-2 IFN-α*	512	512 (100%)‡
IFN-β*	2,048	2,048 (100%)
IFN-γ*	2,048	64 (3.1%)
DS-SLE (whole plasma)	128	16 (12.5%)
DS-SLE (Post-Mono Q)§	192	192 (100%)
DS-SLE (Post-Mono Q/Superose 12)§	256	256 (100%)
DT-SLE (whole plasma)	128	4 (3.1%)
DT-SLE (Post-Mono Q)§	48	48 (100%)

Representative results from multiple experiments (n = 5).
* Samples were diluted in normal human plasma.
‡ Parentheses denote percent activity relative to control dialysis in PBS.
§ IFN-positive fractions were pooled after fractionation and concentrated before assay.
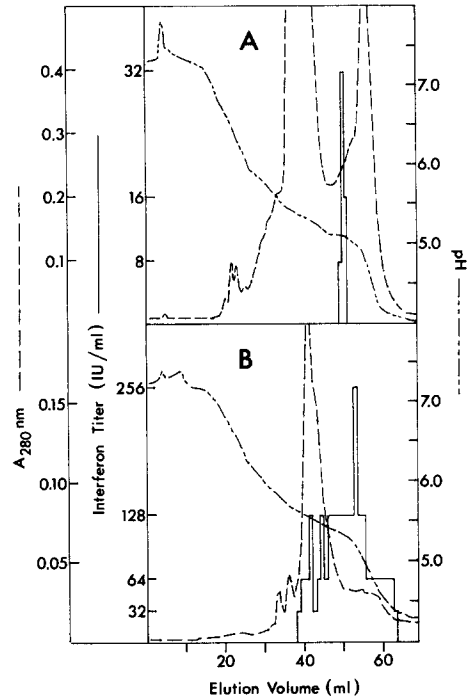
FIGURE 1. FPLC/Mono P chromatofocusing of (A) Mono Q-purified SLE IFN from DS plasma and (B) NK-2 IFN-α. (A) Concentrated Mono Q-purified IFN activity from DS was dialyzed against 25 mM Bis-Tris (Bis[2-hydroxyethyl] imino-tris[hydroxymethyl] methane, pH 7.2) before being fractionated into 1.00-ml fractions on Mono P. The column was equilibrated in 25 mM Bis-Tris (pH 7.2), and samples were eluted with Polybuffer 74 titrated to pH 4.0 (Pharmacia Fine Chemicals) at a flow rate of 0.75 ml/min. (B) Approximately 3 × 10³ IU of NK-2 IFN-α were similarly chromatofocused. Antiviral activity of fractions is illustrated by solid lines (—); absorbance of eluate at 280 nm (A₂₈₀nm) is indicated by evenly broken lines (- - -); pH gradients are denoted by unevenly broken lines (- - — - - —).

unaffected (Table II), suggesting that the apparent acid lability of SLE IFN-α is caused by other factor(s) in SLE plasma that are removed during partial purification.

Acid-dependent IFN-inactivating Factor(s) in SLE Plasma. To test this hypothesis, the effects of SLE plasma on acid-stable NK-2 IFN-α were examined. NK-2 IFN-α was diluted 1:10 (vol/vol) in plasma from either normal or SLE individuals to a final concentration of 512 IU/ml of exogenous IFN activity and was then tested for acid lability. Acid dialysis of NK-2 IFN-α diluted in plasma from DS and DT largely
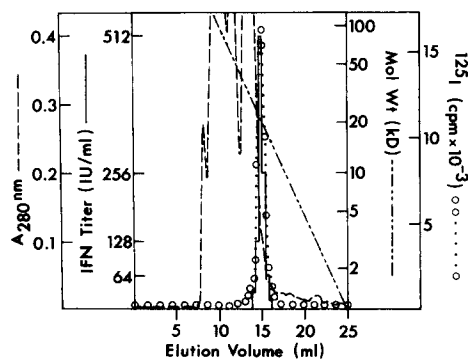

FIGURE 2. FPLC/Superose 12 gel filtration of Mono Q-purified SLE IFN from DS plasma. Mono Q-purified IFN activity from DS was concentrated, dialyzed in PBS, and then fractionated into 0.40-ml fractions at a flow rate of 0.50 ml/min. Antiviral activity of fractions is illustrated by solid lines (—); absorbance of eluate at 280 nm (A₂₈₀nm) is indicated by the evenly broken line (- - -); molecular weight calibration is denoted by the unevenly broken line (— - - — - -). The Superose 12 column was calibrated using the following molecular mass standards: IgG (150 kD), BSA (66.2 kD), OVA (42.7 kD), cytochrome *c* (12.2 kD), and vitamin B₁₂ (1,355 daltons). In parallel experiments, 60 × 10³ cpm of ¹²⁵I-rIFN-α2 were similarly fractionated; radioactivity of individual fractions was quantitated with a gamma counter (· · O · ·).

TABLE III

Acid-dependent Interferon-inactivating Activity

		IFN titer			
		Without added NK-2 IFN-α		With added* NK-2 IFN-α	
Sample	Dialysis buffer:	PBS (pH 7.4)	Glycine-HCl (pH 2)[‡]	PBS (pH 7.4)	Glycine-HCl (pH 2)
		IU/ml		*IU/ml*	
Normal plasma #1		<2	<2	512	512 (100%)[§]
Normal plasma #2		<2	<2	512	512 (100%)
DS-SLE (whole plasma)		256	48 (19%)	768	192 (25%)
DS-SLE (flow-through)[‖]		<2	<2	512	128 (25%)
DS-SLE (salt eluate)[‖]		<2	<2	512	512 (100%)
DT-SLE (whole plasma)		128	4 (3.1%)	512	24 (4.7%)

Representative results from multiple experiments (n = 3).

* The final concentration of added NK-2 IFN-α was 512 IU/ml.

‡ Acid dialysis resulted in >98% inactivation of IFN-γ diluted in normal plasma.

§ Parentheses denote percent activity relative to control dialysis in PBS.

‖ Separately pooled unbound and bound IFN-negative fractions from FPLC/Mono Q of plasma from DS (see text).

inactivated the bulk of both exogenous and endogenous IFN activities (Table III). Additional experiments reveal that 8 of 13 samples from other SLE patients also exhibit similar acid-dependent IFN-inactivating activity and that the presence of such activity is independent of the levels of endogenous IFN (data not shown). No normal plasma showed this property (Table III).

To further test the idea that the acid lability of SLE IFN-α is due to the effects of factors not intrinsic to the IFN, the IFN-negative fractions recovered during Mono Q anion-exchange chromatography of plasma from DS were assayed for acid-dependent IFN-inactivating activity. Fractions from the column flow-through and from the salt eluate which lacked IFN activity were pooled separately and concentrated to original sample volume. The column flow-through, but not the salt eluate, inactivated NK-2 IFN-α after acid dialysis (Table III), suggesting that factor(s) not adsorbing to the Mono Q column at pH 7.5 may account for the apparent acid lability of SLE IFN-α.

Discussion

Four lines of evidence have been advanced in this report to suggest that SLE IFN-α is physicochemically very similar to known IFN-α and that its acid lability is not an intrinsic property of the SLE IFN-α, but rather the result of other factor(s) present in SLE plasma. First, with respect to antigenicity, size, and isoelectric point, SLE IFN-α is indistinguishable from conventional IFN-α. Second, when SLE IFN-α is partially purified by anion-exchange chromatography, it no longer exhibits acid lability. Third, when exogenous acid-stable IFN-α is diluted in SLE plasma or serum, it as well as the endogenous IFN is inactivated after acid dialysis. And last, an acid-dependent IFN-inactivating activity can be partially purified from SLE plasma by anion-exchange chromatography.

These observations have important implications for the study of the IFN system in SLE. Much effort has been expended to clone the large family of human IFN-α

genes and pseudogenes, none of which encode proteins that are acid labile (14). Since it is now shown that SLE IFN-α is not intrinsically acid labile, it is quite possible that one or more of the already described IFN-α may represent the IFN-α subspecies observed in SLE. Thus, the necessary reagents for the study of the IFN system in SLE may already be available for use. Indeed, various subspecies of IFN-α have been shown to stimulate polyclonal B cell activation (15), to inhibit Ts cell function (16), and to modulate expression of HLA class I antigens (17), all of which have been implicated in the induction of autoimmunity. Alternatively, IFN-α may be involved in the efferent limb of pathology in SLE. Experimental and therapeutic administration of IFN-α have been shown to cause clinical signs and symptoms of SLE-like illness in animals and in humans (18).

The significance of acid-dependent IFN-inactivating factor(s) and whether this activity is an integral component of the IFN system in SLE remain to be elucidated. It has been reported that the acid stability of serum IFN-α in AIDS and AIDS-related complex (ARC) decreases with progression of disease, perhaps secondary to the increasing concentration of serum factors such as immunoglobulins, immune complexes, and autoantibodies during the course of illness (8). This speculation may have relevance in SLE, since anti-IFN-α antibodies have been described in some SLE patients (19). However, the nature of acid lability of IFN-α in AIDS/ARC also has not been established, and it would be of interest to determine if findings with acid-labile IFN-α in AIDS/ARC will be similar to those presented here for SLE. Although a change in the acid stability of serum IFN-α in SLE over time has been reported (4), no data on any relationship between acid stability and clinical disease are available. In view of the increasingly recognized similarities between AIDS/ARC and SLE (7), such a study may shed light on possible common pathogenetic or pathological pathways in these disorders. Work to identify the acid-dependent IFN-inactivating factor(s) is currently in progress.

Summary

The physicochemical properties of apparently acid-labile IFN-α from patients with SLE have been studied. The antigenicity, apparent molecular size, and isoelectric point of SLE IFN-α are indistinguishable from those of conventional, previously characterized, acid-stable subspecies of IFN-α. However, after partial purification by anion-exchange chromatography, SLE IFN-α no longer exhibits acid lability, suggesting that other plasma factor(s) are responsible for the acid lability of SLE IFN-α. Addition of SLE plasma, but not normal plasma, to conventional acid-stable IFN-α renders the exogenous IFN-α acid labile. Preliminary results demonstrate that an acid-dependent IFN-inactivating activity can be partially purified from SLE plasma by anion-exchange chromatography.

References

1. Lengyel, P. 1986. On the recent excitement in interferon research. *In* UCLA Symposium on Interferons as Cell Growth Inhibitors and Antitumor Factors. R. M. Friedman, T. Merigan, and T. Sreevalsan, editors. Alan R. Liss, Inc., New York, xxi.

2. Hooks, J. J., H. M. Moutsopoulos, and A. L. Notkins. 1980. The role of interferon in immediate hypersensitivity and autoimmune diseases. *Ann. NY Acad. Sci.* 350:21.

3. Preble, O. T., R. J. Black, R. M. Friedman, J. H. Klippel, and J. Vilček. 1982. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte (α) interferon. *Science (Wash. DC).* 216:429.

4. Hooks, J. J., G. W. Jordan, T. Cupps, H. M. Moutsopoulos, A. S. Fauci, and A. L. Notkins. 1982. Multiple interferons in the circulation of patients with systemic lupus erythematosus and vasculitis. *Arthritis Rheum.* 25:396.

5. Arvin, A. M., and J. J. Miller, III. 1984. Acid labile α-interferon in sera and synovial fluids from patients with juvenile arthritis. *Arthritis Rheum.* 17:582.

6. DeStefano, E., R. M. Friedman, A. E. Friedman-Kien, J. J. Goedert, D. Henriksen, O. T. Preble, J. A. Sonnabend, and J. Vilček. 1982. Acid-labile leukocyte interferon in homosexual men with Kaposi's sarcoma and lymphadenopathy. *J. Infect. Dis.* 146:451.

7. Kopelman, R. G., and S. Zolla-Pazner. 1988. Association of human immunodeficiency virus infection and autoimmune phenomena. *Am. J. Med.* 84:82.

8. Buimovici-Klein, E., M. Lange, R. J. Klein, M. H. Grieco, and L. Z. Cooper. 1986. Long-term follow-up of serum-interferon and its acid-stability in a group of homosexual men. *AIDS Res.* 2:99.

9. Feldmann, M., M. Londei, and G. Buchan. 1987. Interferons and autoimmunity. *In* Interferon 9. I. Gresser, editor. Academic Press, London. 75.

10. Tan, E. M., A. S. Cohen, J. F. Fries, A. T. Masi, D. J. McShane, N. F. Rothfield, J. G. Schaller, N. Talal, and R. J. Winchester. 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 25:1271.

11. Yip, Y. K., R. H. L. Pang, C. Urban, and J. Vilček. 1981. Partial purification and characterization of human γ (immune) interferon. *Proc. Natl. Acad. Sci. USA.* 78:1601.

12. Urban, C., and M. R. J. Salton. 1983. Immunochemical analysis of *Micrococcus lysodeikticus (luteus)* F_1-ATPase and its subunits. *Biochim. Biophys. Acta.* 724:230.

13. Zur Nedden, D. L., and K. C. Zoon. 1986. Radiolabeling of human interferon alphas with ^{125}I-labeled Bolton-Hunter reagent. *Methods Enzymol.* 119:276.

14. Pestka, S. 1986. Interferon from 1981 to 1986. *Methods Enzymol.* 119:3.

15. Harada, H., K. Shioiri-Nakano, M. Mayumi, and T. Kawai. 1983. Distinction of two subtypes of human leukocyte interferon (IFN-α) on B cell activation. *J. Immunol.* 131:238.

16. Knop, J., R. Stremmer, C. Neumann, E. De Maeyer, and E. Macher. 1982. Interferon inhibits the suppressor T cell response of delayed-type hypersensitivity. *Nature (Lond.).* 296:757.

17. Greiner, J. W., P. B. Fisher, S. Pestka, and J. Schlom. 1986. Differential effects of recombinant human leukocyte interferons on cell surface antigen expression. *Cancer Res.* 46:4984.

18. Bottomley, J. M., and J. L. Toy. 1985. Clinical side effects and toxicities of interferon. *In* Interferon, Vol. 4. In Vivo and Clinical Studies. N. B. Finter and R. K. Oldham, editors. Elsevier Science Publishers B. V., Amsterdam. 155–180.

19. Panem, S., I. J. Check, D. Henriksen, and J. Vilček. 1982. Antibodies to α-interferon in a patient with systemic lupus erythematosus. *J. Immunol.* 129:1.