# SERUM INTERFERON LEVELS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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**Levels of interferon (IFN) were measured in 81 serum samples from 23 patients with systemic lupus erythematosus (SLE) by a plaque-reduction method and correlated retrospectively with clinical records of disease activity, anti-DNA binding, and serum complement measurements. IFN titers were found to correlate with both clinical disease activity and anti-DNA binding, but no relation was found to serum complement. Most (76.6%, 31 of 41) serum samples obtained during periods of active disease contained measureable amounts of IFN, but only 9.1% (2 of 22) of results of tests on samples obtained during periods of disease quiescence were positive ($P < 0.005$). Of samples with clearly elevated anti-DNA binding (> 40%), 69.7% (23 of 33) had positive results for IFN, but 57.1% (8 of 14) had negative results when the anti-DNA binding was normal (< 20%) ($P < 0.005$). Measurement of serum IFN titers in patients with SLE, therefore, provides another serologic marker of disease activity. Contrary to the findings of previous studies, the IFN found in the present study was characterized as IFN-$\alpha$, or Type I IFN, on the basis of acid stability and neutralization by antibody to IFN-$\alpha$. Of interest are the questions raised about the origin of IFN in the sera of patients with SLE and what role IFN might have in the pathogenesis of the autoimmune disease in view of the many documented immunomodulating effects of IFN.**

Interferon (IFN) was originally described in 1957 as a glycoprotein, produced by cells infected by virus and able to exert an antiviral effect on other cells (1). This substance has come to be known as Type I IFN, and has been recognized to be induced by pharmacologic agents and bacterial lipopolysaccharide in addition to viral infection (2). A second, closely related antiviral substance was subsequently discovered to be produced by lymphocytes in response to stimulation by nonspecific mitogens (3). This material, Type II IFN, can also be produced upon stimulation of sensitized lymphocytes by specific antigens or antigen-antibody complexes (4). Type I IFN has recently been subclassified as IFN-$\alpha$ if produced by leukocytes or IFN-$\beta$ if produced by fibroblasts. Type II IFN is now termed IFN-$\gamma$ (5).

In addition to antiviral effects, immunoregulatory effects have been described for both types of IFN (4,6). In relation to antibody formation, high doses of IFN have been shown to be suppressive, but low doses enhance antibody production (7–9). Other effects of IFN on elements of the immune system include: inhibition of memory cell formation (10); inhibition of delayed-type hypersensitivity (11) and the graft-versus-host reaction (12); prolongation of allograft survival (13); and enhancement of natural killer (NK) cell activity (14–17) and antibody-dependent cell-mediated cytotoxicity (ADCC) (16,17). Interferon has also been shown to increase the expression of cell surface histocompatibility antigens (18) and to en-

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hance the expression of receptors for the Fc fragment of IgG on T cell surfaces (19). Recently, other investigators have shown IFN to be produced by mitogen-stimulated suppressor T cells, and have postulated that IFN mediates the suppressor activity of these cells (20). Thus, considerable evidence exists that IFN is actively involved in the immune system, since it is produced by antigenic stimulation and is capable of modulating diverse immune responses. The specific physiologic role(s) IFN has in the control of the immune response is, however, unknown.

The observation of the close involvement of IFN with the immune system prompted this investigation of IFN levels in the sera of patients with systemic lupus erythematosus (SLE), a disease characterized by alterations in the immune system. In similar investigations by two other groups, authors have documented elevated levels of IFN in sera of patients with a range of rheumatic disorders, including SLE (21–23). In only one study, however, could a correlation between IFN levels and disease activity in patients with SLE be demonstrated (21,22). In this present investigation, we have analyzed the correlation between serum IFN and several parameters of disease activity, including antibodies to DNA and complement levels, and have begun to characterize the type of IFN present in SLE serum.

## MATERIALS AND METHODS

**Patient selection and serum preservation.** All patients included in this study were treated at the Arthritis Clinic of the University of Michigan Medical Center and met at least 4 of the preliminary criteria of the American Rheumatism Association for the classification of SLE (24). An effort was made to locate patients with active SLE. Patient charts were reviewed to determine clinical disease activity and other laboratory data (the reviewer of the charts had no knowledge of the IFN titers).

Activity was classified as grade 0 if there was no evidence of active disease noted in the chart, or grade 1 if it could not be determined with assurance by either the initial examiner or chart reviewer that active disease was present. Patients with active disease were subclassified as having mild disease (grade 2) if one organ system showed evidence of activity (stable renal disease was not considered a sign of activity). Patients were classified as having severe disease (grade 4) if they had at least two organ systems involved, had been admitted to the hospital for more intensive therapy, or had had their prednisone dose significantly increased. Patients who were classified as having moderate disease (grade 3) had disease severity rated between the previous grade 2 and grade 4 groups. Although this is not a quantitative scale of disease activity, it was devised to account for the fact that it is possible on chart review to differentiate between levels of active disease in patients with SLE. Control sera from 5 patients with osteoarthritis and 6 healthy volunteers were also examined. Serum specimens were stored from the time of collection until assay at −70°C.

**Interferon assay.** A microtiter plaque-reduction assay for interferon was used that is based on the ability of IFN to cause a linear, dose-dependent cellular resistance to viral infection (25). Serum dilutions were made with Dulbecco's modification of Eagle's minimum essential medium (DMEM) supplemented with 2% newborn calf serum (NCS). Human amnion (WISH) cells were grown in microtiter plates as targets for the virus. Diluted serum, 0.1 ml, was added in triplicate to the cell monolayers and incubated overnight at 37°C in 5% $CO_2$. The medium was then removed, and the cells were washed 3 times with DMEM–2% NCS. Between 50 and 75 plaque-forming units of vesicular stomatitis virus-Indiana serotype (VSV) were added per well and allowed to adsorb for 1 hour at 37°C; 0.1 ml of 2% methylcellulose was then added, and the cells were incubated at 37°C in 5% $CO_2$ for 48 hours. The cells were fixed with formalin, stained with Giemsa, and examined under a dissecting microscope for plaques. The IFN activity, in units/ml, was defined as the reciprocal of the highest dilution of serum able to reduce the number of plaques at least 50% as compared to controls. Controls contained no sera, 2% NCS, or known amounts of standard IFN. When titered by this assay system, $10^5$ units of NIH reference human leukocyte IFN #G-023-901-527 yielded results between $3.2 \times 10^4$ and $6.4 \times 10^4$ units (n = 20).

**Demonstration of the antiviral activity to be interferon.** To demonstrate species specificity, samples were assayed on mouse L cell monolayers in addition to WISH monolayers by the technique outlined above for WISH cells. Further evidence that the observed antiviral activity was due to IFN and not to nonspecific antiviral activity was obtained by using neutralization assays with rabbit antibody to human

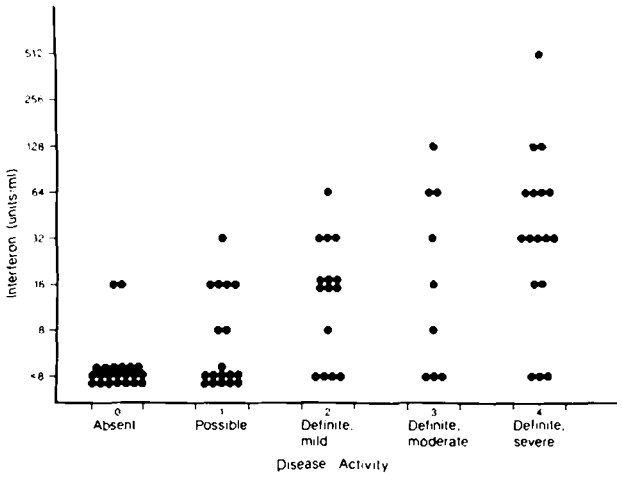


**Figure 1.** Relationship of IFN levels and disease activity. Disease activity was determined by chart review and classified as absent activity (0), possible or indeterminate activity (1), and definitely active disease, subclassified as mild (2), moderate (3), or severe (4) on the basis of the clinical description and physician action ($P < 0.005$, chi square; $r = 0.62$, $P < 0.01$).

IFN-$\alpha$ obtained from the Antiviral Substances Program of the National Institute of Allergy and Infectious Diseases. This antibody was raised toward Sendai virus-induced human leukocyte IFN-$\alpha$ and was purified by immunoabsorption. It has a titer of 1:10,000 toward human IFN-$\alpha$ with a titer of only 1:240 toward human foreskin IFN-$\beta$. The antibody has also been shown to contain titers of less than 1:24 toward antigens from human leukocyte extracts, gammaglobulin, and albumin. Briefly, 0.1 ml of various antibody dilutions in DMEM–2% NCS were incubated with 0.1 ml of positive serum samples at 1 dilution below the end-point titer for 1 hour at 37°C. Samples were then handled according to the standard IFN assay as outlined above. Acid stability was examined by dialyzing aliquots of positive serum samples overnight at 4°C against baths of phosphate-buffered saline titrated to pH 2 or pH 7 with HCl. The samples were then redialyzed against pH 7 buffer and assayed with the standard IFN assay (21).

**Statistical methods.** Chi square analysis was used to test the significance of positive IFN titers versus disease activity, anti-DNA binding, and serum complement with 4, 4, and 1 degrees of freedom, respectively. Additional analysis of the data to compare actual IFN titers and levels of disease activity was done with a linear correlation coefficient analysis, and changes of levels in individual patients were compared by means of the sign test.

## RESULTS

Eighty-one serum samples were analyzed from 23 patients with SLE (1–9 samples per patient). Positive samples were defined as those containing $\geq$ 8 U/ml of IFN. Overall, 70% (16/23) of patients with SLE had at least 1 positive IFN titer, and 49% (40/81) of the serum samples analyzed had positive results. Sera from 5 patients with osteoarthritis and 6 healthy volunteers had negative results for IFN. When the data were analyzed in relation to disease activity (Figure 1), 75.6% (31/41) of samples collected from patients during periods of definite disease activity had positive results, whereas only 9.1% (2/22) of samples obtained during periods of disease quiescence had positive results. In 18 instances, the presence or absence of disease could not be determined with certainty by chart review; 38.9% (7/18) of these samples had positive IFN test results. These data are statistically significant ($P < 0.005$, chi-square; r = 0.62, $P < 0.01$). The relationship of IFN titers to disease activity of individual patients was also analyzed. Comparing the IFN titers from the periods with the greatest and least disease activity within the same patient, the IFN titers varied directly with disease activity in 10 patients, were unchanged in 4 patients, and varied inversely with disease activity in no patients ($P < 0.05$, sign test).

**Table 1.** Relationship of serum interferon and anti-DNA binding activities*

| Anti-DNA binding (%) | Interferon | | | | Total |
|---|---|---|---|---|---|
| | Negative | | Positive | | |
| | No. | % | No. | % | |
| 0–20 | 8 | 57.1 | 6 | 42.9 | 14 |
| 20–40 | 22 | 68.8 | 10 | 31.2 | 32 |
| 40–60 | 6 | 50.0 | 6 | 50.0 | 12 |
| 60–80 | 4 | 33.3 | 8 | 66.7 | 12 |
| 80–100 | 0 | 0.0 | 9 | 100.0 | 9 |

* $P < 0.005$, chi square.

The relationship of the presence of positive IFN titers and anti-DNA binding activities of individual serum samples from all patients studied is shown in Table 1. Of patients with normal anti-DNA binding activities (defined as < 20%), 57.1% (8/14) had negative IFN titers. However, 69.7% (23/33) of patients with clearly elevated anti-DNA binding activities (defined for this purpose as > 40%), had elevated IFN titers ($P < 0.005$). The relationship of IFN titers and complement values is not statistically significant. When the serum complement was normal (C3 $\geq$ 80 mg/dl or CH50 $\geq$ 104 units), 56.3% (18/32) of samples gave negative results for IFN, but 62.5% (25/40) of IFN titers were elevated when the complement was depressed ($P > 0.10$).

Two specific case studies were chosen to illustrate the fact that IFN titers relate most closely to the clinical manifestation of disease, often correlating better with disease activity than with the accepted serologic markers (anti-DNA binding and serum complement).

Patient JW had the highest IFN titer measured, and her course is depicted in Figure 2. She originally came to our clinic at age 44 with a 3-year history of SLE and an explosive flare of her disease 1 month after her prednisone dose had been rapidly tapered and discontinued. At that time, she had joint involvement, rash, pancytopenia, oral ulcers, weakness, fatigue, and anorexia. Proteinuria and hematuria were present, and focal membranous and proliferative glomerulonephritis was shown by a renal biopsy. Results of serologic studies included a C3 of 27 mg/dl and an anti-DNA binding activity of 93.6%. During the ensuing year she was treated with prednisone and hydroxychloroquine with gradual improvement of her symptoms and serologic markers. During this period, her IFN titer dropped from 512 U/ml to less than 16 U/ml.

Figure 3 depicts the course of NZ, a 17-year-old woman followed for approximately 2 years. When first

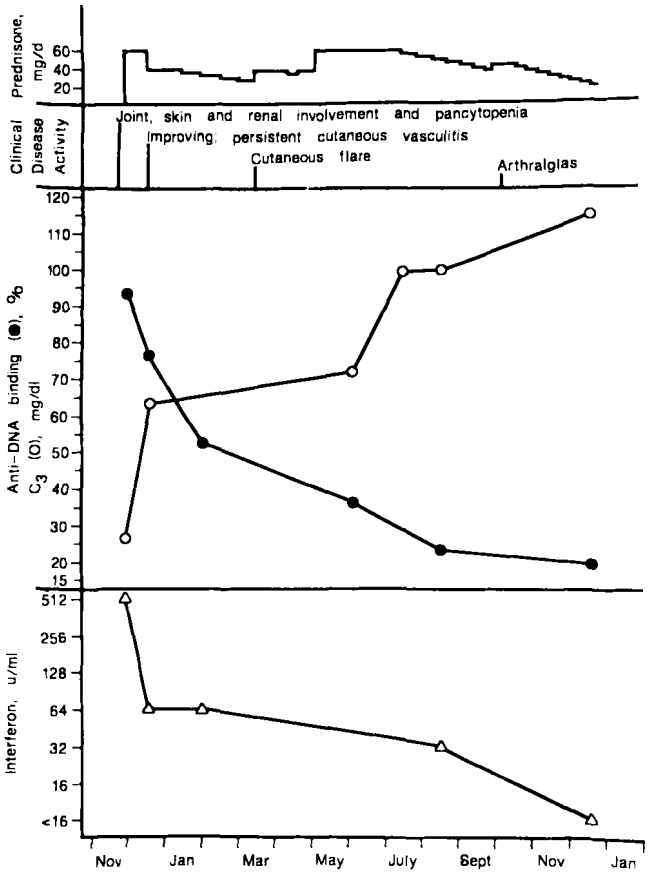


**Figure 2.** Clinical course and laboratory data of patient JW.

seen, she was recovering from a flare of her disease manifested as polyserositis. She subsequently experienced further flares, with skin and renal changes, during which anti-DNA binding activity remained in the normal range while total hemolytic complement was depressed. Analysis of IFN titers in samples obtained during this period showed that the IFN levels rose, correlating with the clinical disease activity rather than with the anti-DNA binding levels.

That the antiviral activity in the sera of the patients studied was due to IFN and not a nonspecific antiviral factor was confirmed by demonstrating species specificity by means of a lack of antiviral effect with mouse L cell monolayer targets. Furthermore, incubation of positive serum samples with VSV did not cause direct neutralization of the virus.

In an attempt to characterize the IFN present, 10 positive samples were treated with acid since IFN-$\alpha$ and IFN-$\beta$ are stable at pH 2, but IFN-$\gamma$ is labile (21). Only 1 of the samples analyzed in this fashion lost antiviral activity after dialysis at pH 2. This 1 sample had a low titer (16 U/ml), and examination of a sample with a higher titer from the same patient showed the IFN to be stable at pH 2. A sample of IFN-$\gamma$ produced by stimulation of normal human mononuclear cells with concanavalin A lost all antiviral activity (initial titer 128 U/ml) after acid dialysis. Further confirmation of the type of IFN present was made by demonstrating complete neutralization of the antiviral activity of 4 of the positive samples by rabbit antibody to human IFN-$\alpha$. Similar complete neutralization of a standard human lymphoblastoid IFN-$\alpha$ was also documented.

## DISCUSSION

The present study confirms the data previously reported by Hooks et al (21,22) and Osial et al (23), who documented the presence of IFN in the sera of patients with SLE. Detectable levels of IFN were found in the sera of 16 of 23 patients with SLE. When analyzed in regard to clinical disease activity, the data confirm the correlation between IFN levels and disease activity: 75.6% of samples obtained when patients had clinically active disease had positive test results, but only 9.1% of samples collected during

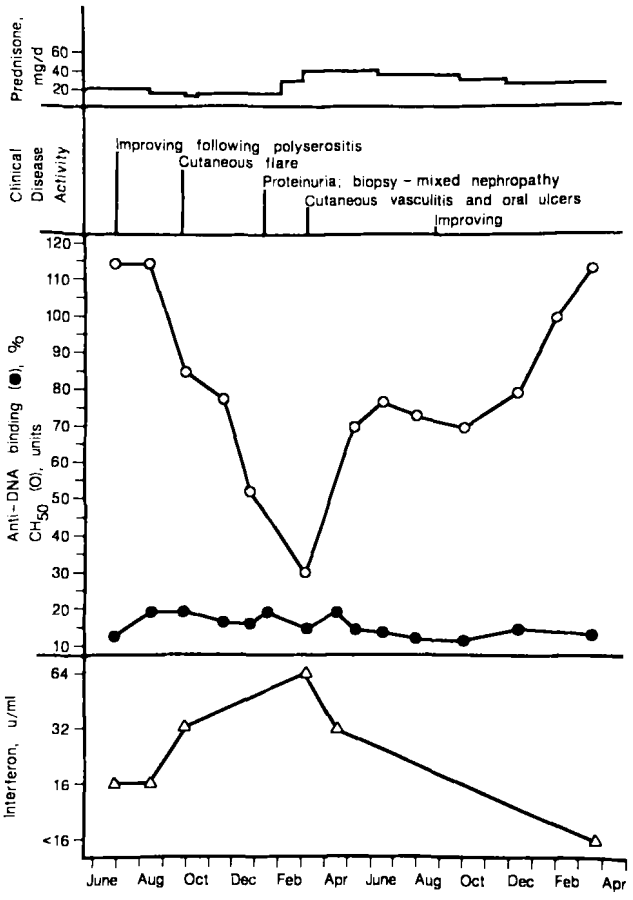


**Figure 3.** Clinical course and laboratory data of patient NZ.

periods of disease inactivity yielded positive results. This correlation is in agreement with the findings of Hooks et al (21,22). Contrary to previous reports, however, in this study the antiviral activity was shown to be stable at pH 2, and therefore more likely to be due to IFN-$\alpha$ rather than IFN-$\gamma$. Neutralization of the antiviral activity by antibody to human IFN-$\alpha$ confirmed that the IFN present was IFN-$\alpha$. The concomitant presence of IFN-$\gamma$ in these samples at levels below those of the IFN-$\alpha$ cannot be entirely ruled out, however. Acid dialysis alone would fail to reduce IFN-$\alpha$ titers, so that any reduction in the lower levels of IFN-$\gamma$ present would not be apparent on the plaque reduction assay. The observation of complete neutralization of IFN activity by the antiserum to IFN-$\alpha$ strongly suggests that either no appreciable amount of IFN-$\gamma$ was present or that the antiserum has cross-reactivity with IFN-$\gamma$.

The data obtained in this report were also examined with respect to two generally accepted laboratory parameters of SLE disease activity, anti-DNA binding and complement levels. A significant relationship to anti-DNA binding activity was found; 69.7% of sera had elevated IFN levels when the anti-DNA binding was $> 40\%$, but 57.1% gave negative results when the anti-DNA binding was $< 20\%$. With respect to serum complement levels, no statistically significant relationship was found. Study of individual patients confirmed that the IFN titers correlated more closely with the clinical rather than the serologic manifestations of disease activity. The relatively poor correlation of IFN titers with anti-DNA binding activity and complement levels is not necessarily an unexpected finding. The role of any individual serologic marker in the pathogenesis of the clinical disease activity of SLE is not well defined, and no serologic parameter has yet been found to correlate perfectly with disease activity (26). Each of the molecules measured as a serologic marker is produced independently and is probably removed at a different rate by different clearance mechanisms. Therefore, the time course of each marker during the disease process must be different, and independent serologic parameters would be expected to correlate better with disease activity rather than with each other.

The finding of elevated IFN titers in the blood of patients with SLE, be it IFN-$\alpha$ or IFN-$\gamma$, is of considerable interest. IFN is known to be intimately linked to the immune system; it is produced by both antigenic and mitogenic stimulation of lymphocytes as well as viral infection, and it is able to modulate a host of immune responses (2–4,6–20). The origin of the IFN in the blood of patients with SLE is unclear, but several possible etiologies exist: 1) Although there has been much investigation into the possible viral origin of SLE, little evidence to support a viral etiology exists to date, making it unlikely that the IFN measured in these patients results from viral stimulation. 2) The elevated IFN levels may result from the presence of circulating immune complexes. Immune complexes are known to be able to stimulate lymphocytes to secrete IFN (4). 3) Since antigenic stimulation of sensitized lymphocytes has been shown to be sufficient to cause production of IFN (4), serum IFN in patients with SLE may be a consequence of the same antigenic stimulation that results in the formation of autoantibodies. 4) Elevated IFN levels may be present as a homeostatic attempt to control immune function in patients with SLE. Patients with SLE are known to have diminished NK cell activity (27), and IFN has been shown to stimulate NK activity (14–17). Similarly, IFN can diminish antibody production in response to antigenic stimulation (7), and its presence may represent a homeostatic attempt to blunt autoantibody formation.

The possibility that IFN itself might be responsible for some or all of the immune aberrations or may be a mediator of the clinical disease expression in SLE must be considered. These hypotheses are supported not only by the known immunomodulatory effects of IFN in humans, but also by observations made in the NZB and NZB/W murine systems. Several investigators have employed IFN or IFN inducers in these mice in order to abrogate the naturally occurring autoimmune disease activity (28–32). Contrary to what had been expected, however, in the majority of these studies, increased autoimmune disease activity and death occurred at an earlier age than usual, suggesting that IFN may play a direct role in mediating the pathophysiology of the autoimmune disease activity.

At this time, measurement of serum IFN titers in patients with SLE provides another serologic marker of disease activity, but one that may not be any more specific than other currently available tests. However, much information remains to be discovered concerning the physiologic roles of IFN in the immune system. Because of the known immune aberrations in SLE, the finding of elevated levels of IFN in the sera of these patients is particularly important and requires further clarification to determine whether it plays a prominent role in the pathogenesis of the disease process or is merely an epiphenomenon. Further work will be necessary to 1) define the cellular origin of IFN in these patients and the stimulus that causes its formation, 2) document the effects of pharmacologic intervention on IFN levels and its correlation with

other immunologic variables in patients with SLE, and 3) investigate the possibility of inhibiting or removing IFN to alter specific immune functions or the disease process.

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