PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **178,** 407–411 (1985)

Inactivation of Interferon by Serum and Synovial Fluids[1] (42024)

P. O'KELLY, L. THOMSEN, J. G. TILLES, AND T. CESARIO

Division of Infectious Diseases and the Department of Medicine, University of California, Irvine, 101 City Drive South, Orange, California 92668

Abstract. The stability of interferons-α, -β, and -γ (IFN) in serum and synovial fluids were compared. Interferon-β was the least stable interferon species, losing at least 75% of its activity during incubation in samples of both serum and synovial fluid. Interferon-α was the most stable IFN, but interferon-γ under these conditions approached the stability of the α species. The inactivation of IFN-β was found due to dialyzable factors and hence not classical antibodies.

Recent publications have reported the presence of interferon (IFN) in the serum (1–3) and synovial fluid (SF) (4, 5) of patients with rheumatic diseases. In these reports, interferon activity has been found more often in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and scleroderma. Detection of interferon, since it seemingly occurs in selected circumstances, may have diagnostic implications.

If IFN detection is to be of diagnostic importance, it would be valuable to know the stability of IFN in the body fluids of patients with rheumatic diseases. This is of special significance in diseases like SLE since it has been reported that such patients can have antiinterferon antibodies (6).

In the past we have shown that serum from normal patients has an adverse effect on IFN-β but not IFN-α (7). In the initial phase of the investigation reported here, we compared the effects of serum from normal controls and from patients with RA, SLE, and degenerative joint disease (DJD) on the bioactivity of IFN-α and -β. Likewise, we tested the effects of SF from patients with rheumatic diseases on the titers of these same interferons. In the second phase of this study we compared the ability of the two body fluids from similar patients to inactivate IFN-γ.

We have found serum and SF from patients with rheumatic disease almost universally inactivated IFN-β but never decreased the titer of IFN-α. Crude IFN-γ was inactivated by a limited number of serum and SF samples from patients with rheumatic diseases. The extent of IFN-α, -β, and -γ inactivation seen in serum from patients with rheumatic diseases was not significantly different from that seen in the same fluid from normal individuals.

Materials and Methods. *IFN assays.* IFN of the α and β subtypes were assayed on foreskin fibroblasts using a microtiter assay as previously described (8). This assay method employs vesicular stomatitis virus (VSV) as challenge. Interferon-γ was assayed on muscle skin fibroblasts (MSF cells) using the same microtiter method except that encephalomyocarditis virus (EMC) was employed as the challenge. The EMC virus was the gift of Dr. I. Braude, Malloy Laboratories (St. Louis, Mo.). All IFN specimens assayed have been standardized using IFN reference α G 023-901-527.

Serum. Serum samples were collected after informed consent from patients on the wards or in the clinics of the University of California Irvine Medical Center and the Long Beach Veterans Administration Hospital. All serum samples utilized were first assayed for endogenous IFN before being tested for ability to inactivate exogenously added IFN. Five of the serum samples were found to contain IFN activity, but in all cases, the titer of IFN present was less than two dilutions (25%) of the final titer after addition of exogenous IFN.

Synovial fluid. SF specimens were obtained from patients whose attending physicians felt diagnostic or therapeutic arthrocentesis was indicated. All patients had active rheumato-

[1] Supported by a grant from the KROC Foundation.

0037-9727/85 $1.50

logical diseases. Fluid was obtained only after appropriate quantities were allocated for clinical testing. All SF specimens used were also assayed for IFN before the inactivation experiments were performed. Only one SF sample had a low titer of IFN present before testing and this titer was well below the level of IFN activity after the addition of exogenous IFN.

IFN preparation. Crude IFN-α, -β, and -γ were prepared in our laboratory as previously reported (9). Partially purified interferon-γ was the generous gift of Dr. I. Braude.

IFN body fluid interaction. For the inactivation experiments, three parts of either SF or serum were mixed with one part of individual IFN preparations. Incubation was carried out in a water bath for 24 hr at 37°C. Concurrently an identical IFN preparation was added to L-15 tissue culture media supplemented with 250 u/penicillin, 150 μg/ml streptomycin, 30 μg/ml glutamine, 90 μg/ml arginine, and 1 mg/ml of glucose. This latter preparation served as the control. After incubation, all samples were immediately titered for IFN activity. IFN residual activity in the body fluid was then calculated as:

$$\text{Residual Activity} = \frac{\text{IFN Titer in Body Fluid}}{\text{IFN Titer in L-15 Diluent}} \times 100.$$

In addition we evaluated the number of specimens inactivating at least two dilutions (75%) of the IFN activity as compared to the control. Specimens inactivating a given IFN species to this extent were termed significant inactivators. The two dilution assessment was selected for statistical analysis because this amount of difference is significant in our microtiter assay and cannot be explained by test variability alone.

Results. In the initial phase of this study we demonstrated that IFN-α and -γ will lose 50% of their activity when incubated at 37°C for 24 hr as compared to an identical aliquot incubated at 4°C. Under these same circumstances IFN-β loses 65% of its activity. Thus to determine inactivation of IFN by body fluids, it is necessary to adjust for thermal inactivation at 37°C. This can be done by relating results to IFN concurrently exposed to control fluids at 37°C.

Table I depicts the results of experiments in which the relative stability of IFN-α and -β in serum are compared. IFN-α was stable (i.e., had identical activity to the control) in every specimen tested. In contrast, 23 out of 30 serum specimens inactivated IFN-β by at least two dilutions of activity (i.e., activity in serum was at least two wells less than that of the controls). This difference is highly significant ($P < 0.01$). The specimens from the patients with rheumatic disease had effects on both IFN-α and -β that were similar to the effects of serum from normals.

All SF tested from both patients with DJD and RA also failed to affect IFN-α activity. These samples, however, adversely influenced IFN-β activity by at least two dilutions in 19 of 20 cases tested. This difference is also highly significant ($P < 0.01$).

In the second stage of the study we tested a series of samples previously examined for ability to reduce IFN-α and -β titers for capacity to influence IFN-γ activity. Since interferon-γ in our laboratory is assayed in a different cell line we wished to be sure the two assay systems gave similar results. We therefore tested serum from five normal patients for ability to inactivate interferon-α, -β, and -γ. These specimens were concurrently incubated and tested on both types of fibroblasts (i.e., IFN-α and -β were assayed in both FF and MSF cells). In every case there was agreement within one dilution between the results in the two cell lines. The normal serum had no influence on α activity (mean RA of 95%) but in all cases inactivated IFN-β by at least two dilutions (mean RA of 7%). Only one of the five specimens inactivated the γ activity by two or more dilutions (mean RA = 76%). A separate group of five additional serum samples from normal controls were also tested for ability to inactivate the three IFN species. After the specimens were appropriately aliquoted, each of the three IFN species was added independently to vials of the serum. Appropriate L-15 controls were prepared for each IFN species. Concurrent incubation was carried out and then titration performed on the appropriate cell lines (IFN-α and -β titrated in FF cells and γ on MSF cells). Of this second control group, all inactivated IFN-β by two or more dilutions and none influenced the activity of

TABLE I. THE EFFECT OF SERUM AND SYNOVIAL FLUID FROM PATIENTS WITH RHEUMATIC DISEASES ON INTERFERON-α AND -β

Diagnosis	Total specimens tested	IFN-α			IFN-β		
		Number specimens with no effect on IFN	Numbers specimens inactivating ≥75% IFN	Mean* residual activity	Number specimens with no effect on IFN	Number specimens inactivating ≥75% IFN units	Mean[a] residual activity
		Serum					
Degenerative joint disease	8	8	0	100%	0	7	24%
Rheumatoid arthritis	15	15	0	100%	1	10	34%
Systemic lupus	7	7	0	100%	1	6	18%
		Synovial fluid					
Degenerative joint disease	8	8	0	100%	0	8	17%
Rheumatoid arthritis	12	12	0	100%	1	11	23%

[a] Mean residual activity after 24 hr incubated at 37°C $= \frac{\text{Interferon Titer in Specimen}}{\text{Interferon Titer in L-15 control}} \times 100.$

either IFN-α or -γ. The mean RA for IFN-α, -β, and -γ, respectively, in the 10 control samples was 95, 16, and 80%. These results were repeated on two occasions.

Subsequently, 12 samples of serum from patients with rheumatic diseases and 13 of samples of SF from similar individuals were examined for IFN-γ inactivation. Two of the serum and two of the SF samples inactivated interferon-γ by two or more dilutions. The two serum specimens included one of three samples from patients with DJD, and one of four samples from patients with RA. Of these two serum samples both had previously been shown to inactivate IFN-β by two dilutions and all had been shown to be without effect on IFN-α. Using the χ^2 method, the number of serum specimens that inactivated IFN-γ

TABLE II. THE EFFECT OF SERUM FROM NORMAL PATIENTS ON INTERFERON-α, -β, AND -γ

Serum specimen	Interferon titer					
	Interferon-α		Interferon-β		Interferon-γ	
	Test	Control	Test	Control	Test	Control
A[a]	320	320	10	160	80	80
B[a]	320	320	20	160	<10	80
C[a]	320	320	10	160	80	80
D[a]	160	320	10	160	40	80
E[a]	320	320	10	160	80	80
F	160	160	10	40	80	160
G	160	160	10	40	160	160
H	160	160	10	40	160	160
I	160	160	10	40	160	160
J	160	160	10	40	160	160

[a] Concurrently titered on both FF and MSF cell lines.

TABLE III. THE EFFECT OF SERUM AND SYNOVIAL FLUID FROM PATIENTS WITH RHEUMATIC DISEASES ON INTERFERON-γ

Diagnosis	Total specimens tested	Number specimens with no effect on IFN	Number specimens inactivated ≥75% of the IFN units	Mean[a] residual activity
		Serum		
Degenerative joint disease	3	0	1	50%
Rheumatoid arthritis	5	1	1	55%
Systemic lupus	4	1	0	63%
		Synovial fluid		
Degenerative joint disease	6	2	2	58%
Rheumatoid arthritis	7	3	0	71%

[a] Mean residual activity after 24 hr incubation at 37°C = $\frac{\text{Interferon Titer in Specimen}}{\text{Interferon Titer in } L_{15} \text{ Control}} \times 100$.

by two or more dilutions was not significantly different than the number inactivating IFN-α ($P > 0.10$), but the number of serum specimens inactivating IFN-β to this extent was significantly different than the numbers inactivating either IFN-α or -γ ($P < 0.05$).

The two SF samples that significantly inactivated interferon-γ were both from patients with DJD and both had been shown to adversely influence IFN-β and to be without effect on IFN-α. Thus the number of SF specimens inactivating IFN-β by two or more dilutions was significantly greater than the number inactivating IFN-α or -γ ($P < 0.05$) but the difference in the number of specimens inactivating IFN-γ to this extent was not significantly different than the number inactivating IFN-α ($P > 0.10$).

Ten samples of serum including 4 from patients with DJD and 6 from patients with RA were exposed to partially purified IFN-γ. None of these 10 samples had any effect on the partially purified IFN-γ nor did 8 samples of SF (4 from patients with DJD and 4 from patients with RA).

Four samples of SF (two from patients with RA and two from patients with DJD), were concurrently tested at 4 and 37°C for interferon-β inactivators. All four samples caused the loss of two or more dilutions, of IFN activity after 24 hr at 37°C as compared with that of the control incubated under the same circumstances. None of the four samples tested demonstrated IFN-β inactivation at 4°C compared with the control samples incubated at the same temperature. Thus, the SF IFN inactivator is temperature dependent.

Aliquots of six SF specimens were dialyzed for 24 hr against PBS and then dialyzed and undialyzed specimens were exposed to IFN-β under the circumstances described above. In five cases, dialysis removed the inactivators as demonstrated by the fact exposure to the undialyzed specimens resulted in the loss of at least two dilutions of IFN activity but exposure to the dialyzed specimens failed to lower the IFN titers. One specimen of dialyzed SF did significantly lower the activity of the added IFN.

Only one of the three serum samples found to significantly inactivate IFN-γ was available for further testing. This sample taken from a normal control was tested before and after dialysis for ability to inactivate IFN-γ. In this situation, dialysis failed to remove the inactivator. Sufficient sample was not available for further characterization of the substance diminishing the IFN-γ activity.

Discussion. These studies clearly demonstrate the greater stability of IFN-α in the serum and SF of patients with rheumatic diseases as compared with that of IFN-β. This is in agreement with earlier studies (10) in various body fluids from normal pa-

tients where the relative stability of IFN-α was initially demonstrated. Furthermore the overall effect of serum from patients with rheumatic diseases was very similar to that of normal patients as regards these two IFN species. In the earlier study 52 of 59 samples adversely influenced IFN-β by at least two dilutions, and the overall MRA was 18%. In this study 23 of 30 samples of serum from patients with rheumatic diseases had a two-dilution adverse influence on IFN-β and the overall MRA was 28%. SF has not been previously studied but seems to behave as serum with a definite effect on IFN-β but not IFN-α.

We have not previously studied the stability of IFN-γ in body fluids, but it appears to resemble more closely the stability of the α rather than the β species. Since the IFN-γ testing was done subsequent to that for α and β, the results are not strictly comparable, but data from the five controls suggest that the assay methods yield similar results and that interferon-β is the least stable species in serum and in SF.

We conclude from this that inactivators of interferons found in serum and SF of patients with rheumatic diseases are unlikely to affect recovery of IFN-α, an acid-labile form of which is the species recovered most often from serum (11) of patients with rheumatic diseases. In contrast these inactivators can regularly influence the bioactivity of IFN-β, the species found least often in samples from such patients. It is possible these inactivators could explain some of the difficulty finding IFN-β in these specimens. It is also possible that in a minority of patients the presence of IFN-γ could also be obfuscated by these inactivators.

Although the number of specimens tested was small, it did not appear that IFN inactivators would be found more often in specimens obtained from patients with any particular disease. Serum specimens from patients with DJD, RA, and SLE all contained β and γ inactivators.

The nature of the inactivators has not been specifically defined, however, in serum these substances are dialyzable factors (10). In the six SF samples tested here the results suggest that this is also the case for arthrodial fluids as dialysis eliminated the inactivator in five of the six samples tested. The results of the dialysis experiments suggest that the inactivators are not antibodies. It is possible that the loss of IFN-β activity represents a tendency to aggregate in these body fluids. We are currently attempting to define specifically the nature of the β inactivators found so frequently in body fluids and the character of the γ inactivators found in a limited number of specimens. It is important to note that the inactivators of the IFN-β species can be prevented from exerting their effect any further by prompt refrigeration of specimens.

1. Hooks J, Moutsopoulos H, Geis S, Stahl N, Decker J, Notkins A. Immune interferon in the circulation of patients with autoimmune disease. N Engl J Med **301**:5–8, 1979.
2. Hooks J, Jordan G, Cupps T, Moutsopoulos H, Fauci A, Notkins A. Multiple interferons in the circulation of patients with systemic lupus erythematosus and vasculitis. Arthritis Rheum **25**:396–399, 1982.
3. Ytterberg S, Schnitzer T. Serum interferon levels in patients with systemic lupus erythematosus. Arthritis Rheum **25**:401–406, 1982.
4. Cesario T, Andrews B, Martin D, Jason M, Treadwell T, Friou G, Tilles J. Interferon in synovial fluid and serum of patients with rheumatic disease. J Rheum **10**:647–650, 1983.
5. Degue M, Mellbye O, Clarke-Jenssen O. Immune interferon in serum and synovial fluid in rheumatoid arthritis and selected disorders. Ann Rheum Dis **42**: 672–676, 1983.
6. Panem S, Check I, Henriksen D, Vilcek J. Antibodies to alpha interferon in a patient with systemic lupus erythematosus. J Immunol **129**:1–3, 1982.
7. Cesario T. The effect of body fluids on polynucleotide induced fibroblast interferon and virus induced leukocyte interferon. Proc Soc Exp Biol Med **144**:1030–1035, 1977.
8. Cesario T, Tillis JG. Inactivation of human interferon by urine. J Infect Dis **127**:311–314, 1973.
9. Cesario T, Schryer P, Tilles J. Relationship between the physicochemical nature of human interferon, the cell induced, and the inducing agent. Antimicrob Agents Chemother **11**:291–298, 1977.
10. Cesario T, Vaziri N, Slater L, Tilles J. Inactivators of fibroblast interferon in human serum. Infect Immun **24**:851, 1979.
11. Preble O, Black R, Friedman R, Kleppel J, Vilcek J. Systemic lupus erythematosus: Presence in human serum of an unusual acid labile leukocyte interferon. Science (Washington, D.C.) **216**:429–431, 1982.

Received April 10, 1984. P.S.E.B.M. 1985. Vol. 178.
Accepted November 14, 1984.