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The Effect of Body Fluids on Polynucleotide-Induced Fibroblast Interferon and Virus-Induced Leukocyte Interferon (39855)

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Recent evidence has suggested that a number of differences exist between human interferon induced in leukocytes by Newcastle Disease Virus (WBC-NDV) and human interferon induced in fibroblasts by a complex of polyinosinic and polycytidylic acids (MSF-IC). Dissimilarities described between these human interferons have included differences in antigenicity (1), in circumstances of renaturation (2), in sensitivity to heat (3), and in sensitivity to guanidine (4).

In the past we have described the ability of various human body fluids (5, 6) to inactivate MSF-IC. Since it is now evident that MSF-IC and WBC-NDV are different, it is of interest to compare the effects of such fluids on the two interferons.

In this report the effect of human cerebrospinal fluid, saliva, serum, urine, or bile has been determined, simultaneously, on samples of MSF-IC and WBC-NDV. It has been found that WBC-NDV is significantly more resistant to the effects of cerebrospinal fluid, saliva, serum, and urine. This information has important implications relative to the application of human interferon in disease states and to the understanding of the pharmacology of interferon in the body.

Materials and methods. Preparation of virus-induced human interferon in white blood cells (WBC-NDV). After separation by low-speed centrifugation from blood acidified with ammonium chloride for 10 min, human white cells were washed twice with Leibovitz medium (L-15). The cells were subsequently suspended at a density of 10 million/ml in L-15 supplemented with 5% fetal calf serum (FCS) and rolled in test tubes for 18 hr at 37° in the presence of Newcastle Disease Virus (multiplicity of 100 EID50 per cell). The interferon-containing medium was separated by pelleting the cells at low-speed centrifugation and then sequentially dialysing against 0.1 *M* citric acid at pH 2.8 for 5 days and phosphate-buffered saline (PBS) at pH 7.4 for 24 hr. After centrifugation at 25,000 rpm for 90 min in a Beckman SW 27 rotor, 2-ml aliquots of the pooled interferon containing supernatant were frozen at −80°.

Preparation of poly(I:C)-induced human interferon in MSF Cells (MSF-IC). Human muscle-skin fibroblasts (MSF) were exposed to 10 μg/ml of Poly(I:C) and 100 μg/ml of DEAE-D (MW 2,000,000) for 3 hr, washed three times, and incubated overnight in L-15 supplemented with 5% FCS. The interferon containing-medium was then harvested and stored at −80° in 2.0-ml aliquots.

Interferon assays. Samples of interferon incubated in CSF, saliva, and serum were assayed in a previously described (5, 7) microtiter method which is performed in muscle skin fibroblasts (MSF cells) and uses vesicular stomatitis virus (VSV) as a challenge. Samples incubated in urine or bile were assayed by a plaque reduction (5) technique detailed elsewhere which also employs MSF cells and VSV.

Human body fluids. Samples of cerebrospinal fluid were obtained from the clinical laboratories. With two exceptions, noted in Table I, all CSF samples were clear and had normal white blood cell counts, sugar, and protein values. These samples were stored at 4° for variable periods ranging from 3 to 46 days prior to testing. Twelve saliva samples from 11 normal adults were Millipore-filtered to achieve sterility and stored at 4° for periods of 0 to 10 days before testing.

Serum samples were separated from freshly clotted blood obtained from 11 healthy adults. Subsequent storage was at 4° for periods of 2 to 62 days.

To six urine samples obtained from five adults, penicillin, 250 units/ml, and streptomycin, 150 μg/ml, were added, and the testing was performed immediately.

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Bile samples, obtained intraoperatively from four patients undergoing biliary tract surgery, were stored at 4° for periods ranging from 7 to 21 days.

Body fluid-interferon interaction. The studies were performed by adding one part of interferon-containing tissue culture media to three parts of body fluid. Simultaneous controls were prepared by incubating one part of interferon in three parts of L-15 tissue culture medium containing 150 units/ml of penicillin, 250 μg/ml of streptomycin, 1 μg/ml of glucose, 30 μg/ml of glutamine, and 90 μg/ml of arginine. In the experiments performed using bile, interferon was diluted 1:1 with the bile in order to permit greater dilution of the test mixture and hence avoid tissue culture toxicity from the bile. Similarly bile controls were diluted 1:1 in L-15 medium.

After appropriate dilution, incubation was carried out in a water bath at 37°. Cerebrospinal fluid, saliva, and serum were incubated for 24 hr and urine and bile for 4 hr.

Statistical significance. The residual activity remaining after incubation in each body fluid sample was calculated according to the following formula: Residual activity = (interferon titer in body fluid/interferon titer in L-15 media) × 100. For each body fluid the number of specimens having a greater effect on MSF-IC was compared to the number of specimens with a greater effect on WBC-NDV by the Wilcoxon matched pairs signed rank test. In addition mean residual activities were compared by Student's *t* test.

Results. Preliminary experiments demonstrated that after 4 hr at 37° both MSF-IC and WBC-NDV interferons lose 50% of their original activity when compared to controls similarly incubated at 4°. After 24 hr at 37°, WBC-NDV interferon loses no further activity, but the activity of MSF-IC interferon falls to 34.4% of the original. Thus, to determine inactivation of interferon by human body fluid it is necessary to adjust for thermal inactivation at 37°. This can be done by relating results to interferon concurrently exposed to control fluids at 37°.

Table I records the effect of 10 specimens of cerebrospinal fluid on MSF-IC and WBC-NDV interferons. Each of the spinal fluids adversely affected MSF-IC interferon but only two of the ten specimens affected WBC-NDV. In virtually every case the spinal fluids tested caused a greater activity loss with MSF-IC than with WBC-NDV.

TABLE I. Residual Activity of Human Interferon after 24 Hours of Incubation in Cerebrospinal Fluid.

	MSF-IC			WBC-NDV			
Specimen	Sample titer	Control titer	Residual activity[a] (%)	Sample titer	Control titer	Residual activity[a] (%)	*p*
1	12[b]	96	12.5	48	48	100	
2	24	384	6.3	>48	48	100	
3	48	384	12.5	48	48	100	
4	6	96	6.3	192	192	100	
5[c]	6	24	25.0	24	48	50.0	
6	48	192	25.0	12	24	50.0	
7	48	192	25.0	24	24	100	
8[d]	48	192	25.0	24	24	100	
9	12	24	50.0	>24	24	100	
10	6	24	25.0	>24	24	100	
Mean			21.3%			90.0%	
Significance							
Student's *t*							<0.01
Wilcoxon matched pairs[e]							<0.01

[a] Residual activity (RA) = (interferon activity in cerebrospinal fluid/interferon activity in L-15 diluent) × 100.

[b] Units/0.025 ml.

[c] Abnormal fluid with 14 leukocytes, normal glucose and protein values.

[d] Abnormal fluid with 625 leukocytes, normal glucose and protein values.

[e] Wilcoxon matched pairs: number ^{RA}MSF-IC < ^{RA}WBC-NDV = 10; number ^{RA}MSF-IC > ^{RA}WBC-NDV = 0.

The difference in the number of specimens having a greater effect on MSF–IC with the number having a greater effect on WBC–NDV is significant ($p < 0.01$). Similarly, the difference in the mean residual activities of MSF–IC (21.3%) and WBC–NDV (90%) is significant ($p < 0.01$).

Table II demonstrates the effect of saliva on the two interferons. While MSF–IC lost activity when exposed to virtually every specimen, WBC–NDV was resistant to three of the 12 samples tested. When the number of saliva specimens having a more adverse influence on MSF–IC is compared to the number having a more adverse influence on WBC–NDV, the difference is significant ($p < 0.05$). However, the difference in mean residual activities of MSF–IC and WBC–NDV is not significant ($p > 0.20$).

The effect of serum on the two interferons is shown in Table III. Of the 11 serums tested all but one partially inactivated MSF–IC, but only three of the 11 adversely affected WBC–NDV activity. When the number of serum specimens causing a greater activity loss for MSF–IC is compared to the number causing a greater loss for WBC–NDV, the difference is significant ($p < 0.01$). Similarly, the difference in mean residual activities of MSF–IC and WBC–NDV is significant ($p < 0.01$).

The ability of urine to decrease interferon activity is shown in Table IV. While all samples at least partially inactivate both interferons, the reduction in activity is greater for MSF–IC than WBC–NDV for every sample tested ($p < 0.05$). The difference in mean residual activities for MSF–IC and WBC–NDV is also statistically significant ($p < 0.05$). In like manner, the effect of bile is clearly greater on MSF–IC than WBC–NDV, although the number of samples is insufficient for a statistical analysis.

Discussion. The application of interferon to the treatment of human disease has met with only limited success. Both topically and systemically administered interferons must be used in very large doses to achieve therapeutic benefit (8, 9), and it is possible that inactivation of interferon by body fluids is a contributing factor. Most of the clinical trials reported have utilized WBC–NDV. Since MSF–IC interferon has physicochemical differences from WBC–NDV, the latter could prove to be more efficacious clinically. However, the present study documents that MSF–IC is more sensitive to inactivation *in vitro* by cerebrospinal fluid, serum, saliva, urine, and bile.

TABLE II. Residual Activity of Human Interferon after 24 Hours of Incubation in Saliva.

	MSF–IC			WBC–NDV			
Specimen	Sample titer	Control titer	Residual activity[a] (%)	Sample titer	Control titer	Residual activity[a] (%)	p
1	6[b]	24	25.0	≤1.5	12	≤12.5	
2	12	96	12.5	12	48	25.0	
3	12	24	50.0	24	48	50.0	
4	12	24	50.0	24	48	50.0	
5	≤3	24	≤12.5	24	24	100	
6	6	24	25.0	24	24	100	
7	≤3	24	≤12.5	12	24	50.0	
8	≤12	48	≤25.0	24	48	50.0	
9	96	96	100	96	192	50.0	
10	6	96	6.3	48	192	25.0	
11	12	48	25.0	96	192	50.0	
12	12	24	50.0	24	24	100	
Mean			32.8%			55.2%	
Significance							
Student's t							>0.20
Wilcoxon matched pairs[c]							<0.05

[a] Residual activity (RA) = (interferon activity in saliva/interferon activity in L-15 diluent) × 100.
[b] Units/0.025 ml.
[c] Wilcoxon matched pairs: number ^{RA}MSF–IC < ^{RA}WBC–NDV = 8; number ^{RA}MSF–IC > ^{RA}WBC–NDV = 2.

TABLE III. Residual Activity of Human Interferon after Incubation in Serum.

	MSF-IC			WBC-NDV			
Specimen	Sample titer	Control titer	Residual activity[a] (%)	Sample titer	Control titer	Residual activity[a] (%)	p
1	6[b]	24	25.0	≤1.5	12	≤12.5	
2	24	192	12.5	24	24	100	
3	24	384	6.3	12	24	50.0	
4	24	192	12.5	24	24	100	
5	3	48	6.3	24	24	100	
6	24	24	100	>48	48	100	
7	6	48	12.5	6	12	50.0	
8	12	48	25.0	12	12	100	
9	6	48	12.5	>24	24	100	
10	12	48	25.0	>24	24	100	
11	24	48	50.0	>24	24	100	
Mean			26.1%			83.0%	
Significance							
Student's t							<0.01
Wilcoxon matched pairs[c]							<0.01

[a] Residual activity (RA) = (interferon activity in serum/interferon activity in L-15 diluent) × 100.
[b] Units/0.025 ml.
[c] Wilcoxon matched pairs: number ^{RA}MSF-IC > ^{RA}WBC-NDV = 1; number ^{RA}MSF-IC < ^{RA}WBC-NDV = 9.

TABLE IV. Residual Activity of Human Interferon after 4 Hours of Incubation in Urine or Bile.

	MSF-IC			WBC-NDV			
Specimen	Sample titer	Control titer	Residual activity[a] (%)	Sample titer	Control titer	Residual activity[a] (%)	p
			URINE				
1	20[b]	>2000	≤1	112	2000	5.6	
2	30	>2000	≤1.5	250	500	50.0	
3	50	>2000	≤2.5	200	500	40.0	
4	200	>2000	≤10	1000	2000	50.0	
5	160	2000	8.0	250	900	27.8	
6	800	2000	40	800	950	84.3	
Mean			6.5%			43.0%	
Significance							
Student's t							<0.05
Wilcoxon matched pairs[c]							<0.05
			BILE				
1	0	>2000	<1	300	1200	25.0	
2	0	1250	<1	0	>2000	<1	
3	30	300	10	2500	2500	100	
4		300	<1	1250	2500	50.0	
Mean			2.5%			44.0	

[a] Residual activity (RA) = (interferon activity in body fluid/interferon activity in L-15 diluent) × 100.
[b] Units/2 ml.
[c] Wilcoxon matched pairs: number ^{RA}MSF-IC < ^{RA}WBC-NDV = 6; Number ^{RA}MSF-IC > ^{RA}WBC-NDV = 0.

While *in vivo* studies will be required to demonstrate the actual importance of the inactivation of interferons by body fluids, it is possible that these inactivation processes will be clinically significant. It would be helpful to know if inactivation can occur in interstitial fluid or even on the surface of cells. The time required for exogenous interferon to reach the target cells might prove to be important. Although such questions can only be answered by *in vivo* studies, the *in vitro* investigations reported here suggest that WBC-NDV, because it is more resistant to inactivation by body fluids, would be better than MSF-IC as a candidate interferon for human use.

Summary. The effects of five human body fluids on human fibroblast interferon (MSF-IC) and human leukocyte interferon (WBC-NDV) are reported. After 24 hr at body temperature, WBC-NDV is significantly more stable than MSF-IC in cerebrospinal fluid, saliva, and serum. After 4 hr at 37°, leukocyte interferon is significantly more stable in urine and appears more stable in bile. These findings may be useful in the choice of an exogenous human interferon for clinical studies.

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