Proc. Nat. Acad. Sci. USA
Vol. 72, No. 6, pp. 2185–2187, June 1975

Two Antigenically Distinct Species of Human Interferon

[poly(I)·poly(C)/paramyxovirus/antibodies against interferon/affinity chromatography]

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Communicated by H. Sherwood Lawrence, March 20, 1975

ABSTRACT **Rabbit antisera prepared against interferon produced in human fibroblast cell cultures stimulated with poly(I)·poly(C) neutralized the activity of interferon preparations produced in various human fibroblast cultures stimulated either with poly(I)·poly(C) or with viruses. However, these antisera showed no detectable neutralizing activity against interferon produced in cultures of human leukocytes. On the other hand, most rabbit antisera against the human leukocyte interferon were active in neutralizing both homologous interferon and fibroblast interferons. A preparation of antiserum against leukocyte interferon, active against both leukocyte and fibroblast interferons, was shown by affinity chromatography to have two distinct antibody populations, one of which was specific for the fibroblast interferon. We conclude that the heterologous neutralizing activity of sera from rabbits immunized with leukocyte interferon is likely to be due to the presence of two antigenic species of interferon. The major antigenic species of leukocyte interferon preparations (designated "Le") is distinct from human fibroblast interferon. The minor species of leukocyte interferon ("F") is either identical with, or closely related to, interferon produced in human fibroblast cultures.**

It was noted that antisera prepared against interferon produced in short-term cultures of human buffy coat leukocytes stimulated with parainfluenza 2 (Sendai) virus showed greater neutralizing activity against homologous interferon than against human interferons from three other cellular sources (1). It was also reported that an antiserum raised against human leukocyte interferon failed to neutralize interferon produced in cultures of human amnion cells (2). These findings suggested that human interferons may be antigenically heterogeneous.

More suggestions for the antigenic heterogeneity of human interferons come from some recent studies. Anfinsen *et al.* (3) prepared a sheep antiserum against human leukocyte interferon for use in affinity chromatography. This antiserum, attached to Sepharose, bound both homologous interferon and heterologous human interferon produced in fibroblast cultures; however, its affinity for the heterologous interferon might have been somewhat lower. Berg *et al.* (4) found that three out of four rabbit antisera against human leukocyte interferon neutralized human fibroblast interferon, although their neutralizing titer was generally lower than against homologous interferon. On the other hand, antisera produced against human fibroblast interferon completely failed to neutralize the activity of leukocyte interferon preparations (4, 5).

This study establishes the antigenic specificities of interferons present in preparations obtained from human leukocyte and fibroblast cultures. It shows that the major interferon species present in these preparations are antigenically distinct and that preparations of leukocyte interferon appear to contain a mixture of two species, the minor component being identical with, or closely related to, fibroblast interferon.

MATERIALS AND METHODS

Interferon Preparations. Interferon was induced in a strain of diploid human foreskin fibroblasts (FS-4) with poly(I)·poly(C), Newcastle disease virus, or β-propiolactone-inactivated Sendai virus (6), as described (7, 8). Human embryonic kidney interferons were the kind gifts of Mr. Ron Weiss (Abbott Laboratories, North Chicago, Ill.), and interferon of Sendai virus-induced human peripheral leukocytes (9) was the generous gift of Dr. Kari Cantell (State Serum Institute, Helsinki, Finland). Spent medium from the human lymphoblastoid cell line 8866, containing interferon produced "spontaneously" by these cells (10), was kindly provided by Drs. Richard Lerner and Steven Kennel of the Scripps Clinic and Research Foundation, La Jolla, Calif.

Interferon Neutralization Assay. Rabbit immunization schedules, route and quantity of interferons administered, and the titration of sera for interferon neutralizing activity have been described (4, 11). The neutralizing titer is defined as the reciprocal of the highest dilution of the antiserum that inhibited the antiviral action of 10 interferon reference units/ml (final concentration), as determined by inhibition of the cytopathic effect of vesicular stomatitis virus in cultures of human fibroblasts.

Binding of Interferon to Sepharose 4B. A total of 1.5×10^5 reference units of poly(I)·poly(C)-induced human foreskin interferon in 15 ml was covalently coupled to 15 ml of packed CNBr-activated Sepharose 4B swollen beads (Pharmacia Fine Chemicals, Uppsala, Sweden) by a modification of the method of Ankel *et al.* (12). Coupling was allowed to occur at 4° while the reaction mixture was slowly rotated for 18 hr. An interferon assay demonstrated 400 units of interferon activity per 0.1 ml of reacted beads. Column fractions were concentrated by ultrafiltration using an Amicon model 12 stirred cell with a PM 10 membrane filter (Amicon Corp., Lexington, Mass.).

RESULTS

The neutralization of leukocyte and fibroblast interferons by antisera prepared against homologous and heterologous interferon preparations is summarized in Table 1. Antisera against leukocyte interferon showed a variable degree of neutralization of the heterologous fibroblast interferon, with the ratio of anti-leukocyte/anti-fibroblast neutralizing activity ranging

TABLE 1. *Distribution of neutralizing antibodies against leukocyte and fibroblast interferons in rabbits immunized with leukocyte or fibroblast interferons*

Antisera against interferon from	Neutralizing titer against interferon from		Anti-leukocyte/ anti-fibroblasts
	Leukocytes*	Fibroblasts†	
Leukocytes* no. 13	24,000	1,500	16
24	9,000	800	11
26	3,000	2,400	1
30	18,000	<100	>180
Fibroblasts† no. 4	<70	1,500	<0.05
5	<70	1,200	<0.06
6	<70	400	<0.2
29	<70	4,500	<0.02

* Interferon induced with Sendai virus.

† Interferon produced in FS-4 cells by poly(I)·poly(C).

TABLE 2. *Neutralization of various human interferons by antisera against fibroblast and leukocyte interferons*

Interferon preparation		Neutralizing titer with serum	
Cell source	Inducing agent	Anti-fibroblast	Anti-leukocyte*
FS-4	Poly(I)·poly(C)	1,400	200
	NDV	1,600	400
	Sendai virus	1,200	300
HEK†	Poly(I)·poly(C)	1,200	300
	NDV	1,200	400
Leukocyte	Sendai virus	≤30	1,200
Lymphoblastoid cell line 8866	None‡	≤64	600

NDV, Newcastle disease virus.

* Lyophilized serum from rabbit no. 26 (see Table 1).

† Cell strain derived from human embryo kidney cells.

‡ Spent growth medium containing interferon produced "spontaneously" without exposure to an inducer.

from about 1 to >180. This variable degree of heterologous neutralizing activity seemed to be determined primarily by the response of individual rabbits because the ratio of the two neutralizing activities remained relatively constant in sera collected from individual rabbits at various times during the immunization process. In marked contrast, none of the sera from four rabbits immunized with the fibroblast interferon neutralized the leukocyte interferon.

To determine whether the nature of the antigenicity of the interferon was the function of the cell source or possibly determined by the nature of the inducing agent, two antisera, prepared against the FS-4 or leukocyte interferons, respectively, were tested against a number of human interferon preparations produced in the FS-4 cells or in human embryo kidney cell cultures by stimulation with poly(I)·poly(C), Newcastle disease virus, or Sendai virus. All of these preparations were indistinguishable from each other in their neutralizability with the two antisera, whereas the Sendai virus-induced leukocyte interferon and 8866 interferon were clearly distinct in their antigenicity (Table 2). We can conclude that it was the producing cell, rather than the type of inducer used, that determined the antigenic specificity of the interferon preparations included in our assay.

There appeared to be two possible explanations for the observation that antisera against the human leukocyte interferon did show a variable degree of neutralization of interferons produced in human fibroblasts. One possible explanation was that interferons from the two different cell sources might share some common antigenic determinants. However, if this were the case, it would be difficult to explain why cross-neutralization occurred only in one direction, i.e., why antisera against the fibroblast interferon failed to show any neutralizing activity against leukocyte interferon. Furthermore, if the two interferons indeed shared an antigenic determinant, the ratios of homologous to heterologous neutralizing activities of various anti-leukocyte interferon sera should probably be less variable than actually found (see Table 1).

The other explanation, which we considered to be more likely, was that leukocyte interferon preparations contain a mixture of two antigenically distinct species of interferon: (*i*) a major component that is specific for leukocyte interferon, and (*ii*) a minor component that is either identical with, or related to, fibroblast interferon.

The latter possibility has recently been suggested by Berg *et al.* (4) on the basis of affinity chromatography studies: when a preparation of leukocyte interferon was passed through a column of Sepharose-bound anti-fibroblast interferon globulin, the bulk of interferon activity did not adsorb to the column. (This result was not unexpected, since the antibody used showed no neutralizing activity against leukocyte interferon, as also demonstrated in Tables 1 and 2.) Of possible significance, however, was the observation that a small fraction (about 4% of the total leukocyte interferon applied to the column containing anti-fibroblast interferon globulin) did adsorb to the column, and was recovered in the eluate obtained with low pH buffer. It was concluded that this minor fraction might represent a component of leukocyte interferon that has the antigenic specificity of fibroblast interferon.

The existence of two antigenically distinct components in leukocyte interferon was further supported by neutralization tests with monospecific antiserum against leukocyte interferon obtained by "reverse" affinity chromatography on a column of human fibroblast interferon covalently bound to Sepharose 4B, as described in *Materials and Methods*. The antiserum chosen was from rabbit no. 26, that showed good neutralizing activity against both leukocyte and fibroblast interferons (compare Tables 1 and 2).

This antiserum was passed through the affinity column and a control column of Sepharose 4B without interferon (Table 3). No significant decrease of anti-leukocyte neutralizing activity occurred after the serum was passed through either the affinity or control column. Antifibroblast interferon activity, on the other hand, decreased to undetectable levels after the passing of serum through the affinity column. The latter activity also decreased significantly on the control column, possibly due to nonspecific adsorption, although antibody to leukocyte interferon was not similarly affected. No activity could be eluted from either column with 0.5 M NaCl solution, pH 7.0. However elution with a buffer consisting of 0.1 M acetic acid and 0.5 M NaCl at pH 2.5 resulted in the recovery of a significant portion of anti-fibroblast interferon neutralizing activity from the affinity column, whereas

TABLE 3. *Neutralization of fibroblast and leukocyte interferons by an antiserum against leukocyte interferon fractionated by affinity chromatography on a column of human fibroblast interferon bound to Sepharose*

Fraction of serum tested for neutralizing activity	Neutralizing titer against interferon from	
	Fibroblasts	Leukocytes
Original serum*	128	192
Void—interferon column†	≤8	128
Void—control column‡	24	192
Eluate 0.5 M NaCl—interferon column†	≤8	≤8
Eluate 0.5 M NaCl—control column‡	≤8	≤8
Eluate pH 2.5 buffer—interferon column†	48	≤8
Eluate pH 2.5 buffer—control column‡	≤8	≤8

* Serum from rabbit no. 26 (see Table 1) after 1:3 dilution in phosphate-buffered saline, pH 7.4.

† Fibroblast interferon covalently bound to Sepharose 4B, as described in *Materials and Methods*.

‡ Sepharose 4B alone.

no anti-leukocyte interferon activity was recovered in the same eluate. No neutralizing activity could be detected in the eluate from the control column.

DISCUSSION

The results of this study indicate that the antiserum against leukocyte interferon contains two populations of neutralizing antibodies specific for leukocyte and fibroblast interferons. The findings also provide strong circumstantial evidence for the notion that leukocyte interferon preparations contain two antigenically distinct interferons, one of which is either identical with, or closely related to, fibroblast interferon.

We propose to designate the major antigenic component of human leukocyte preparations "Le interferon." The antigenic species that apparently forms the minor component of leukocyte preparations (and, at the same time, may represent either the major or exclusive species of human fibroblast interferon) should be designated "F interferon."

If preparations of interferon made in cultures of buffy coat cells were to contain Le and F interferon at a ratio of about 20:1, it would not be surprising that some animals immunized with this material make antibodies against both antigenic types of interferon (with the antibody levels against Le interferon being generally higher than against F interferon), while other animals might produce only anti-Le interferon antibodies. The reason for the presence of two antigenically distinct interferons in preparations obtained from cultures of buffy coat cells could be that more than one of the cell types present in these cultures are involved in interferon production.

It is possible that other, as yet unrecognized, antigenic species of human interferon exist in addition to the Le and F species. Thus, Valle, Jordan, and Merigan (13) have recently found that preparations of human interferon obtained by the immunologically specific stimulation of sensitized lymphocytes (14) contained activity that could not be neutralized by antiserum against Sendai virus-induced leukocyte interferon. Since Valle and coworkers used antiserum from rabbit no. 26 that was also used in our experiments (Tables 1 and 2), their finding suggests that human immune-specific interferon might be antigenically distinct from both Le and F interferons.

Stewart and Desmyter (15) recently showed that electrophoresis of human leukocyte interferon on sodium dodecyl sulfate gels resulted in the separation of interferon activity into two distinct peaks: a major one corresponding to 15,000 daltons and a minor one of 21,000 daltons. It will be of interest to determine if the major and minor peaks found on sodium dodecyl sulfate gels have the antigenic specificity of Le and F interferon, respectively.

We thank Dr. Joel Oppenheim and Lenore Gardner for their useful suggestions and help. We are grateful to Lyn Gradoville and Angel Feliciano for their skilled technical assistance. This investigation was supported by Grant AI-07057 and Contracts NO1-AI-02169 and NO1-AI-02126 from the USPHS. B.B. was supported by Medical Scientist Training Program Grant 2-TO5-GM-01668.

1. Levy-Koenig, R. D., Mundy, M. J. & Paucker, K. (1970) *J. Immunol.* **104**, 785–790.
2. Duc-Goiran, P., Galliot, B. & Chany, C. (1971) *Arch. Ges. Virusforsch.* **34**, 232–243.
3. Anfinsen, C. B., Bose, S., Corley, L. & Gurari-Rotman, D. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3139–3142.
4. Berg, K., Ogburn, C. A. & Paucker, K. (1975) *J. Immunol.* **114**, 640–644.
5. Vilček, J., Havell, E. A., Mozes, L. W. & Berman, B. (1975) *Proc. 1st Intersect. Congr. Internat. Assoc. Microbiol. Soc.*, Tokyo, Sept. 1–7, 1974, in press.
6. Giles, R. E. & Ruddle, F. H. (1973) *In Vitro* **9**, 103–107.
7. Havell, E. A. & Vilček, J. (1972) *Antimicrob. Ag. Chemother.* **2**, 476–484.
8. Vilcek, J. & Havell, E. A. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3909–3913.
9. Strander, H. & Cantell, K. (1967) *Ann. Med. Exp. Biol. Fenn.* **45**, 20–29.
10. Henle, G. & Henle, W. (1967) *Cancer Res.* **27**, 2442–2446.
11. Havell, E. A., Vilček, J., Falcoff, E. & Berman, B. (1975) *Virology* **63**, 475–483.
12. Ankel, H., Chany, C., Galliot, B., Chevalier, M. J. & Robert, M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2360–2363.
13. Valle, M. T., Jordan, G. W. & Merigan, T. C. (1975) *J. Immunol.*, in press.
14. Epstein, L. B., Stevens, D. A. & Merigan, T. C. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2632–2636.
15. Stewart, W. E., II & Desmyter, J. (1975) *Virology*, in press.