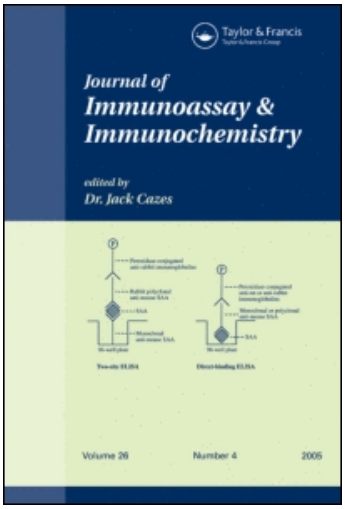

Journal of Immunoassay and Immunochemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Application of Namalva Interferon-α Monoclonal Antibodies for Purification and Enzyme Immnoassay of Interferon-α

Joji Notani[a]; Tsutomu Kaizu[a]; Seitarou Mutoh[a]; Kazuyuki Otsuka[a]; Chihiro Kusunoki[a]; Hisashi Yamada[a]; Akira Nagayoshi[a]; Shingo Suzuki[a]; Mineo Niwa[a]; Ikuo Ueda[b]; Monoclonal Antibody[a]
[a] Research Laboratories, Fujisawa Pharmaceutical, Co., Ltd., Osaka, Japan [b] The Institute of Scientific and Industrial Research, Osaka University, Osaka

To cite this Article Notani, Joji , Kaizu, Tsutomu , Mutoh, Seitarou , Otsuka, Kazuyuki , Kusunoki, Chihiro , Yamada, Hisashi , Nagayoshi, Akira , Suzuki, Shingo , Niwa, Mineo , Ueda, Ikuo and Antibody, Monoclonal(1989) 'Application of Namalva Interferon-α Monoclonal Antibodies for Purification and Enzyme Immnoassay of Interferon-α', Journal of Immunoassay and Immunochemistry, 10: 2, 257 — 276
To link to this Article: DOI: 10.1080/01971528908053240
URL: http://dx.doi.org/10.1080/01971528908053240

JOURNAL OF IMMUNOASSAY, 10(2&3), 257-276 (1989)

APPLICATION OF NAMALVA INTERFERON-α MONOCLONAL ANTIBODIES FOR PURIFICATION AND ENZYME IMMNOASSAY OF INTERFERON-α

Joji Notani, Tsutomu Kaizu, Seitarou Mutoh, Kazuyuki Otsuka, Chihiro Kusunoki, Hisashi Yamada, Akira Nagayoshi, Shingo Suzuki, Mineo Niwa* and Ikuo Ueda**

Research Laboratories, Fujisawa Pharmaceutical, Co., Ltd., Yodogawa-ku, Osaka 532, Japan
** Present address: The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567

*To whom correspondence should be addressed

ABSTRACT

Namalva (or Namalwa) interferon (IFN)-α was partially purified using a combination of conventional methods and modified acid-ethanol extraction. Four mouse monoclonal antibodies against Namalva IFN-α were prepared by hybridoma technology after immunization with Namalva IFN-α thus purified. Three of these monoclonal antibodies recognized the same or a similar epitope on Namalva IFN-α. One of these antibodies was paired with the fourth recognizing a different epitope and used respectively as enzyme-conjugated antibody and solid-phase antibody in our one step enzyme immunoassay (EIA) for IFN-α. This assay is simple and was able to detect as little as 5 pg of IFN-α in 100 μl of sample in the short time of 5 hr. There was a good correlation between the EIA and bioassay. The use of one of the monoclonal antibodies as an immunoadsorbant to purify Namalva IFN-α is also described.

(KEY WORDS: Namalva interferon-α, Purification, Monoclonal antibody, Enzyme immunoassay (EIA), Pharmacokinetics)

INTRODUCTION

Human IFN-α is a lymphokine produced mainly when lymphocytes are incubated with Sendai virus or Newcastle-disease virus. Its properties include the protection of cells against a wide range of viruses,[1,2] inhibition of cell growth,[3,4] modulation of immune response,[3] and enhancement of the synthesis of several cellular proteins.[5] In addition, IFN-α has clinically proven antitumor effects,[6,7] and clinical potential for Kaposi's sarcoma[8,9] in AIDS.

The bioassay of interferon is relatively complex and time-consuming. Consequently, it would be advantageous to be able to measure rapidly and accurately IFN-α concentrations in various experimental and clinical situations. IFN-α levels have been assayed by EIA[10-13] but in these methods monoclonal antibodies against human leukocyte IFN-α have been used. We describe here the preparation of 4 monoclonal antibodies against Namalva IFN-α and the establishment of an EIA system using 2 monoclonal antibodies recognizing different epitopes on IFN-α. This assay is simple and sensitive enough to detect 1 IU/100 µl (5 pg/100 µl) IFN-α in the short time of 5 hr. The variation coefficient was 5-10% depending on the concentration of IFN-α used. We also describe the efficient purification of Namalva IFN-α using one of the monoclonal antibodies.

MATERIALS AND METHODS

Interferons

Lymphoblastid (Namalva) IFN-α was obtained from Namalva cells treated with Sendai virus as described by Strander.[14]

Human IFN-β was obtained from Flow 7000 foreskin fibroblast treated with polyI·poly C, and human IFN-γ from human spleen lymphocytes treated with phytohemagglutinin.

Purification

All procedures were carried out at 4-7°C unless described otherwise. Supernatant from Namalva cells was brought to pH 3.5 by adding hydrochloric acid (HCl) and mixed batchwise with SP Sephadex C-25 (Pharmacia) equilibrated with McIlvine citrate phosphate buffer (pH 3.5). The mixture was filtrated on a sintered glass filter and eluted with McIlvine citrate phosphate buffer (pH 7.8) to give the IFN-α fraction, which was treated with potassium thiocyanate (KSCN) at pH 3.5 as described by Cantell[15] to give the IFN-α precipitate. The precipitate was dissolved in 0.02 M phosphate buffered saline (PBS) containing 0.5% sucrose to obtain a clear solution, and then loaded onto Sephacryl S-200 superfine (Pharmacia) equilibrated with the same buffer. The resulting IFN-α fractions were pooled and partially purified by controlled-pore glass bead adsorption using a CPG-10 (Electro-nucleonics), eluted as described by Whitman Jr.[16], and pooled and treated with KSCN at pH 3.5. The resulting precipitate was resolved and rechromatographed on Sephacryl S-200 superfine as described above, the IFN-α fraction was concentrated to 0.1 mg protein/ml using a YM-10 filter (Amicon), and the residue was adjusted to pH 3.5 with HCl and added dropwise to the ethanol

solution saturated with KSCN under gentle stirring for 2-3 hr at -20°C to obtain IFN-α. The insoluble precipitate was then removed and the supernatant was purified according to Cantell[15] to obtain highly purified IFN-α.

Interferon titration

Interferon was titrated by cytopathic effect inhibition assay using human FL aminion cells infected with vesicular stomatitis virus.[17] All titers were expressed in accordance with international reference standard sample, G023-901-527, supplied by the National Institute of Allergy and Infectious Disease, Bethesda, MD, USA.

Determination of protein concentration

Protein concentration was determined by the method of Lowry[18], with bovine serum albumin (BSA) as a standard.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed with 15% (W/V) polyacrylamide gel slabs using a tris-glycine (pH 8.8) buffer system as described by Laemmli[19] in the presence or absence of 2-mercaptoethanol (2-ME). The gels were stained with coomassie brilliant blue (CBB).

Preparation of monoclonal antibodies

BALB/c mice (Shizuoka Animal Lab. Center) were injected intraperitoneally twice or 3 times with 2.8-3.0 x 10^6 IU of partially purified Namalva IFN-α (2.1 x 10^6 or 2.8 x 10^7 IU/mg

protein), and additionally with 2.0 x 10^9 cells of killed Bordetella pertusis (Research Institute for Microbial Diseases, Osaka University) in some cases. Four and three days before killing the mice, they were given intravenously 2.8 x 10^6 and 1.4 x 10^6 IU of partially purified Namalva IFN-α (2.8 x 10^7 IU/mg), respectively, to fortify the immunization. Immune spleen cells were fused with mouse myeloma P3X63Ag8-U1 (P3U1) after the method of Köhler and Milstein,[20] using 45% polyethyleneglycol 4000 (Sigma) as the fusion reagent. The fused cells were then distributed in 24-well plates (Nunc) in Dulbecco's modified Eagle's medium (Flow Lab.) supplemented with 15% fetal calf serum (Centaurus), 2 mM L-glutamine, 2 x 10^{-5} M 2-ME and antibiotics, and fed on alternate days with selective HAT medium. After feeding for 2 weeks, the supernatants were tested for antibody production described by Secher.[21] Briefly, Namalva IFN-α (1.0 x 10^4 IU) and 0.6 ml of the culture supernatant were mixed and incubated at 37°C for 2 hr. The antibody-antigen complex was precipitated by adding carrier mouse serum and rabbit anti-mouse IgG, incubated at 37°C for 1 hr, and left to stand at 4°C for 15 hr. The resulting solutions were centrifuged to remove the antibody-antigen precipitate and the supernatants were assayed for the residual IFN-α content as described above. Positive cultures were cloned several times by the limiting dilution method in 96-well culture plates. The obtained clone cells were inoculated into the peritoneal cavity of mice primed with 2,6,10,14-

tetramethylpentadecane (Wako Pure Chemicals), and the ascitic fluid containing the monoclonal antibody was harvested 10 to 20 days later. The gamma globlin fraction was purified by ammonium sulfate fractionation and ion-exchange chromatography on DE-52[22] (Whatman).

Preparation of anti Namalva IFN-α Sepharose 4B

Monoclonal antibodies to Namalva IFN-α were coupled to CNBr activated Sepharose 4B (5 mg/ml gel, Pharmacia) by the method of Berg.[23]

Purification of Namalva IFN-α using FAI-1 Sepharose 4B

The Namalva IFN-α partially purified by SP Sephadex C-25 ion-exchange chromatography and KSCN precipitation and Sephacryl S-200 superfine gel filtration was further purified by FAI-1 Sepharose 4B affinity column chromatography as described by Staehelin[24] to give pure IFN-α.

Isolation and purification of Namalva IFN-α by preparative SDS-PAGE

In this step, gel electrophoresis apparatus (Model 1650, Instrumentation Specialities) was used. Electrophoresis was carried out according to described procedures[19] using 4.5%, 14% and 7% slab polyacrylamide gels. The gel combinations were as follows:

Upper gel : 1.5 cm height of 4.5% gel

Middle gel : 4.0 cm height of 14% gel

Lower gel : 5.0 cm height of 7% gel

After setting up the gel, electrophoresis was precycled at 10W for 15 hr. The fraction from FAI-1 Sepharose 4B was mixed with the sample buffer without 2-ME after the method described by Laemmli.[19] During the electrophoresis, protein from the lower gel was eluted with 0.3 M tris-HCl (pH 8.8) containing 0.08% SDS.

Amino acid sequence analysis

The reduced and S-carboxymethylated IFN-α obtained above was sequenced with a Beckman 890 D sequnator[25] and manually converted to PTH amino acid, which was analyzed by HPLC on u-Bondapack C_{18}[26] (Waters) or Ultrasphere ODS[27] (Ultex) columns monitoring at 254 nm and 313 nm.

EIA Procedures

Conjugation of horseradish peroxidase (POD; type IV, Sigma) to monoclonal antibody was performed as described by Nakane and Kawaoi.[28]

EIA was carried out as follows. Antibody 5 μg/ml in 15 mM carbonate buffer (pH 9.6) was allowed to stand at room temperature for 1 hr in 96-well plates (Dynatech Immulon plate). The plates were rinsed 3 times with PBS containing 0.1% BSA, and 100 μl of POD-conjugated monoclonal antibody in PBS containing 5% bovine serum and 100 μl of sample were added to each well and allowed to stand at room temperature for 3 hr. The wells were washed with PBS containing 0.1% BSA and 0.05% Tween 20, and 200 μl of substrate solution (2.5 mg/ml of o-phenylenediamine

dihydrochloride and 0.018% of hydrogen peroxide in citrate phosphate buffer, pH 5.0) was added to the wells and left for 30 min. The reaction was stopped by adding 50 µl of 3 M sulfonic acid. The absorbance was read on a MR micro-Elisa microreader (Dynatech) at 490 nm.

RESULTS AND DISCUSSION

Purification of Namalva IFN-α by a combination of conventional methods and modified acid-ethanol extraction

The purification of Namalva IFN-α is summarized in Table 1. We added a modification of the acid-ethanol extraction developed by Cantell as the last step of the purification. This procedure gave a good overall-recovery but required as many as 7 steps. The specific activity of IFN-α at the last step was 2.8×10^7 IU/mg protein, which is sufficient purity for use in immunization. The specific activity was about 10 times higher than 2.2×10^6 IU/mg protein reported by Cantell.

Screening of hybridomas producing monoclonal antibodies against Namalva IFN-α

Four mice were immunized with the Namalva IFN-α obtained as above. Two mice produced sufficient antibody to neutralize 50% of 100 IU IFN-α, and were used for the cell fusion experiments. Of the 204 cultures obtained in the spleen cell fusion experiment, 4 culture showed good production of the antibody. The fused cells were then cloned several times to obtain stable hybridomas

TABLE 1

Steps in the Purification of IFN-α Leading to Acid-Ethanol Extraction

Steps	Volume (ml)	Units recovered ($\times 10^{-6}$)	Protein recovered (mg)	Specific activity (IU/mg protein)	Recovery (%)
Culture sup.	45×10^3	264	110×10^3	2.4×10^3	100
SP Sephadex C-25	2.4×10^3	216	36.6×10^3	5.9×10^3	82
KSCN ppt	100	206	22.6×10^3	9.1×10^3	78
Sepahcryl S-200(S)	400	200	819	2.4×10^5	76
CPG-10	288	251	434	5.7×10^5	95
KSCN ppt	5	200	348	5.7×10^5	76
Sephacryl S-200(S)	35	145	45	2.1×10^6	55
Acid-ethanol extraction	50	137	4.8	2.8×10^7	52

producing monoclonal antibody against Namalva IFN-α. From this cloning we obtained 4 kinds of hybridomas producing monoclonal antibodies and designated them as FAI-1, FAI-2, FAI-3 and FAI-4. The immunogloblin subclass was determined for each monoclonal antibodies by double immunodiffusion using goat antiserum to mouse immunogloblin subclass (Miles), and FAI-1, FAI-3 and FAI-4 were classified as IgG_1 and FAI-2 as IgG_{2a}.

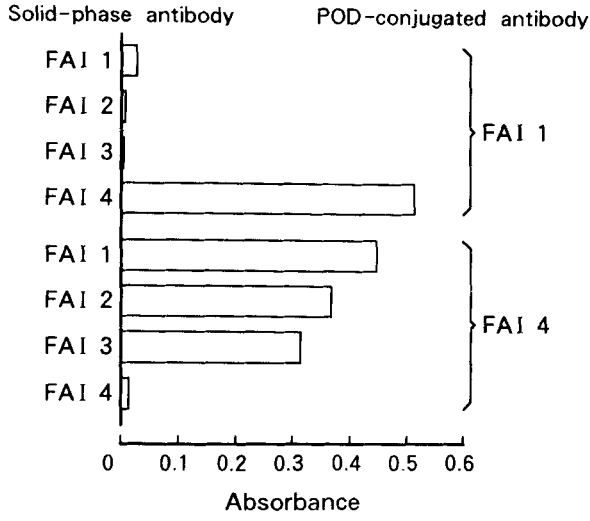

Figure 1. Double antibody EIA using different combinations of monoclonal antibodies and 400 IU/ml of IFN-α. Ninety six well-plates were coated with each monoclonal antibody, and incubated with 400 IU of Namalva IFN-α and POD-conjugated FAI-1 (or FAI-4), and then washed with PBS containing 0.1% BSA and 0.25% Tween 20. The absorbance at 490 nm was measured after incubation with substrate solution for 30 min as described in Materials and Methods.

Epitopes on Namalva IFN-α recognized by monoclonal antibodies

Two kinds of monoclonal antibodies which recognize different antigenic determinants and independently bind to Namalva IFN-α without any mutual interference, would enable us to develop an EIA with high sensitivity. To select this combination, we characterized the four antibodies obtained above as shown in Figure 1. The antibodies were placed in microtiter wells as solid-phase, and IFN-α and the POD-conjugated FAI-1 or FAI-4 antibody were added simultaneously to sandwich IFN-α between the

solid-phase and conjugated antibodies. The binding of the conjugated antibody depended on and was proportional to the IFN-α bound to the solid-phase antibody. After incubation and washing, the IFN-α concentration was determined by measuring the amount of bound POD-conjugated antibody. As shown in Figure 1, the POD-conjugated FAI-1 was able to sandwich Namalva IFN-α effectively with FAI-4, whereas, FAI-1 as well as FAI-2 and FAI-3 were able to sandwich with the POD-conjugated FAI-4. These results indicate that only FAI-4 recognized a different epitope.

Application of FAI-1 Sepharose 4B affinity chromatography and preparative SDS-PAGE for purification of Namalva IFN-α

Monoclonal antibody column chromatography has been used to simplify and bring efficiency to the purification of IFN-α.[21,24,29] FAI-1 Sepharose 4B affinity chromatography would simplify the conventional purification procedure of Namalva IFN-α, which needs as many as 7 steps. Namalva IFN-α partially purified using SP Sephadex C-25, KSCN precipitation and Sephacryl S-200 superfine as described above was applied to this system to give Namalva IFN-α of improved purity of 5.5×10^7 IU/mg protein. The recovery of IFN-α was 70%. 24% of the applied IFN-α was not retained and this may have been due to the lower affinity of the IFN-α molecules than that of the bound population to the monoclonal antibody. When this IFN-α was further analyzed by SDS-PAGE electrophoresis, CBB staining showed various bands including a major one at molecular weight (MW) around 18.5 Kd and

TABLE 2

Application of FAI-1 Sepharose 4B Affinity Chromatography and Preparative SDS-PAGE for Purification of Namalva IFN-α

Steps	Sepcific activity (IU/mg protein)	Recovery (%)
Culture sup.	2.4×10^3	100
SP Sephadex C-25	5.9×10^3	82
KSCN	9.1×10^3	78
Sepahcryl S-200(S)	2.4×10^5	76
FAI-1 Sepharose 4B	5.5×10^7	53
Preparative SDS-PAGE	2.0×10^8	43

an another at about MW 39 Kd, which could be removed by repeated passage through the same column as described by Secher[21] (data not shown). Preparative SDS-PAGE was used to further purify the IFN-α obtained by FAI-1 Sepharose 4B affinity chromatography to obtain IFN-α of 4-fold purity. The SDS-PAGE was carried out under non-reducing condition in order to avoid inactivation of the IFN-α , by using the stepwise gel system as described in Materials and Methods. Namalva IFN-α purified by the SDS-PAGE exerts a specific activity of 2.0×10^8 IU/mg protein and shows a single band at MW 18.5 Kd under non-reducing condition (data not shown). This procedure was more simple than the one shown in Table 1, and

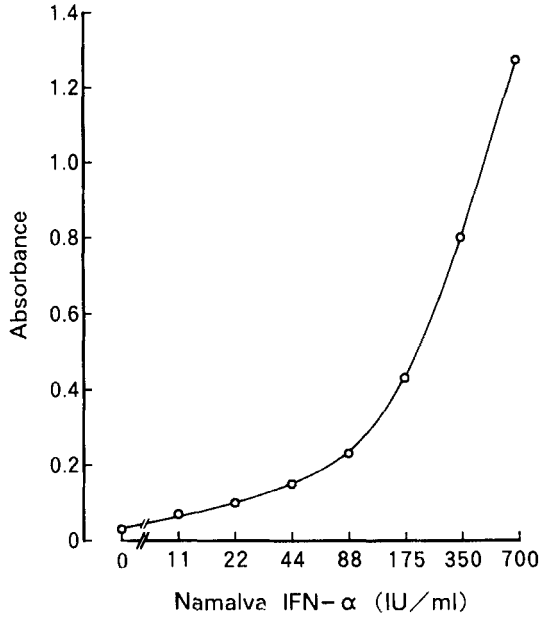

Figure 2. Standard curve for Namalva IFN-α in one-step antibody EIA. FAI-1 and FAI-4 were used as a POD-conjugates antibody and solid-phase antibody respectively. Crude IFN-α (specific activity was approximately 6.5×10^4 IU/mg protein) was used in the assay. Details are given in Materials and methods.

is presented in Table 2. The overall-recovery was good; 43%. The purified IFN-α showed 3 bands including 2 major ones at MW 19 Kd and 19.5 Kd, and a minor one at MW 22 Kd by SDS-PAGE in the presence of 2-ME (data not shown). This suggests that the IFN-α purified by FAI-1 chromatography contained at least 3 of the 8 components reported by others.[30-32] The IFN-α thus purified showed the same amino acid sequence in 21 residues from N-terminal as that of IFN-α_2 reported by Pestka.[33]

TABLE 3

Reactivity[a)] of Monoclonal Antibodies to IFNs

Monoclonal antibodies	Namlva IFN-α	Leukocyte IFN-α	IFN-β
FAI-1	90%	81%	0%
FAI-4	85%	97%	0%

a) 4.8×10^3 IU of Namalva IFN-α, 9.0×10^4 IU of leukocyte IFN-α or 9.0×10^3 IU of IFN-α was loaded onto 0.5 ml of Sepharose 4B coupled with monoclonal antibody, FAI-1 or FAI-4, and the column was washed sufficiently with PBS. Interferons not retained to the column was titrated as described in Materials and Methods. Reactivity (%) = (1-(IFN not retained to monoclonal antibody-Sepharose 4B)/IFN loaded onto the Sepharose 4B column) X 100

One step EIA

EIAs are convenient and reproducible methods for rapid detection of antigens. In this study, an EIA using two monoclonal antibodies was used for detection of Namalva IFN-α. Two monoclonal antibodies, FAI-1 and FAI-4, recognized most of the IFN-α_s produced in leukocyte and lymphoblastid (Namalva) cells, especially IFN-α_2.

Figure 2 shows a standard curve for Namalva IFN-α in one step EIA using FAI-1 and FAI-4 as POD-conjugated antibody and solid-phase antibody, respectively. As little as 10 IU/ml (approximately 5 pg/100 µl sample) Namalva IFN-α was detectable by this EIA in 5 hr.

TABLE 4

	Sample[a)]	n	Mean ± SD (IU/ml)	CV (5)[b)]
Intra-assay	A	10	476 ± 19.8	4.1
	B	10	279 ± 17.3	6.2
	C	10	50 ± 2.6	5.2
	D	10	25 ± 2.7	10.8
	E	10	9 ± 0.9	10.0
Inter-assay	F	5	520 ± 15.1	2.9
	G	5	255 ± 3.3	1.3
	H	5	50 ± 4.3	8.6

a) IFN-α added to human serum
b) % coefficient of variation

Crude Namalva IFN-α was applied to Sepharose 4B coupled with monoclonal antibodies in order to estimate the reactivity of the monoclonal antibodies. As shown in Table 3, FAI-1 and FAI-4 failed to recognize some IFN-α subspecies. This EIA also failed to detect inactivated IFN-α heated at 60°C (data not shown). As expected, human IFN-β and IFN-γ were not detected by this assay.

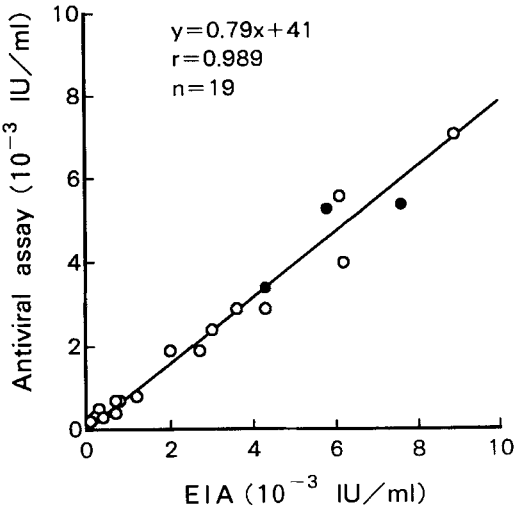

Figure 3. Comparison of EIA and antiviral assay for determination of IFN-α. Details are described in Materials and Methods. (o): Namalva IFN-α, (●): leukocyte IFN-α.

Inter- and intra-assay precision at various IFN-α concentrations added exogenously to human serum to examine whether this EIA was of practical use, are shown in Table 4. Coefficient of variation at different concentrations was less than 10.8% and 8.6% for intra- and inter-assay, respectively, and indicates that this procedure would be useful for measuring IFN-α in the serum.

To examine the correlation between the EIA and bioassay methods, test specimens containing different amounts of IFN-α were measured by both methods. The results (Figure 3) showed good correlation between the two procedures (r= 0.989). This EIA would therefore be much more useful than biological assay from the

standpoint of rapidity, ease of performance and reproducibility. This EIA system would also be useful for measuring IFN-α in clinical samples.

ACKNOWLEDGEMENTS

We would like to thank Prof. T. Watanabe, Kyushu University, Kyushu for supplying us with myeloma cell line and providing the opportunity to study hybridoma technology; Dr. H. Nitta, Kyoto Red Cross Blood Center, Kyoto for providing leukocyte IFN-α ; and Dr. Y. Nagano for helpful suggestions concerning the IFN research program. We also thank Dr. S. Katsuki, Dr. M. Izeki, Miss Y. Kawami, Mr. S. Arakawa, Mr. T. Yamashita, and Mr. S. Osawa for their helpful discussions and assistance.

REFERENCES

1. Nagano, Y., Kojima, Y. and Sawai, Y. Immunité et intérference dans la vaccine. Inhibition de l'infection dermique par le virus inactive. C. R. Soc. Biol. 1954;148:750-752.

2. Issacs, A. and Lindemann, J. Virus interference. 1. The interferon. Proc. Roy. Soc. B. 1957;147:258-267.

3. Stewart-II, W. E. (editor), 1979, The interferon system. Springer-Vertag, Vienna, Austria.

4. Gresser, I, Brouty-Boyé, D., Thomas, M., Macieria-Coelho, A. Interferon and cell divsion, I. Inhibition of the multiplication of mouse leukemia L 1210 cells in vitro by interferon preparations. Proc. Natl. Acad. Sci. USA. 1970;66:1052-1058.

5. Hovanessian, A. G. and Kerr, I. M. The (2'-5')oligoadenylate (pppA2'-5'A2'-5'A) synthetase and protein kinase(s) from interferon-treated cells. Eur. J. Biochem. 1979;93:515-526.

6. Billiau, A. The clinical value of interferons as antitumor agents. Eur. J. Cancer, Clin. Oncol. 1981;17:949-967.

7. Strander, H. Proc. Intern. Symp. Interferons, Kishida, K. (editor), Kyoto, 1983:p151.

8. Real, F. X., Oettgen H. F. and Krown, S. E. Kaposi's sarcoma and the acquired immunodeficiency syndrome:treatment with high and low dose of recombinant leukocyte A interferon. J. Clin. Oncol. 1986;4:544-551.

9. Gelman, E. P., Preble, O. T., Steis, R., Lane, H. C., Rook, A. H., Wesley, M., Jacob, J., Fauci, A., Masur, H. and Longo, D. Human lymphoblastoid interferon treatment of Kaposi's sarcoma in the acquired immune deficiency syndrome. Clinical response and prognostic parameters. Am. J. Med. 1985;78:737-741.

10. Staehelin, T., Stähli, C., Hobbs, D. S. and Pestka, S. A rapid quantitative assay of high sensitivity for human leukocyte interferon with monoclonal antibodies. Methods in Enzymol. 1981;79:589-595.

11. Gallati, H. Interferon: A greatly simplified enzyme-immunological determination, using two monoclonal antibodies. J. Clin. Chem. Clin. Biochem. 1982;20:907-914.

12. Horovitz, O., Novich, D., Rubinstein, M. and Revel, M. Rapid-immunoassays and ELISA for human leukocyte IFN-α . Immunoenzymatic Techniques 1983;18:193-196.

13. Agui, A., Ito, K., Miyata, M., Sekiguchi, S. Enzymeimmunoassay of human interferon-α using monoclonal antibody. Hokkaidou Igaku Zasshi 1985;60:424-433.

14. Strander, H., Mogenson, K. E. and Cantell, K. Production of human lymphoblastoid interferon. J. Clin. Microbiol. 1975;1:116-117.

15. Cantell, K. and Hirvonen, S. Large-scale production of human leukocyte interferon containing 10^8 Units per ml. J. Gen. Virol. 1978;39:541-543.

16. Whitman, J. E. Jr., Crowley, G. M., Tou, J., Tredway, J. V., Hung, C. Purification of human lymphoblastoid cell-derived interferon-alpha by controlled-pore glass bead adsorption chromatography and molecular sieving. J. Interferon Res. 1981;1:305-313.

17. Armstrong, J. A. Cytopathic effect inhibition assay for interferon: Microplate assay. Methods in Enzymol. 1981;78:381-387.

18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951;193:265-275.

19. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 1970;227:680-685.

20. Köhler, G. and Milstein, C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature (London) 1975;256:495-497.

21. Secher, D. S. and Burke, D. C. A monoclonal antibody for large-scale purification of human leukocyte interferon. Nature (London) 1980;285:446-450.

22. Savasti, J. and Milstein, C. The disulphide bridges of a mouse immunogloblin G1 protein. Biochem. J. 1972;126:837-850.

23. Berg, K. Sequential antibody affinity chromatography of human luekocyte interferon. Scan. J. Immunol. 1977;6:77-86.

24. Staehelin, T., Hobbs, D. S., Kung, H., Lai, C., Pestka, S. Purification and characterization of recombinant human leukocyte interferon (IFLrA) with monoclonal antibodies. J. Biol. Chem. 1981;256:9750-9754.

25. Edman, P. and Begg, G. A protein sequenator. Eur. J. Biochem. 1967;1:80-91.

26. Dowing, M., Mann, K. and Elion, J. Improved high-pressure liquid chromatographic separation of amino acid phenylthiohydantoins. J. Chromatogr. 1978;155:436-438.

27. Somach, R. Complete phenylthiohydantoin amino acid analysis by high-performance liquid chromatography on ULTRASPHERE-Octadecyltrimethyloxysilane. Anal. Biochem. 1980;104:464-468.

28. Nakane, P. K. and Kawaoi, A. Heterogenous enzyme immunoassay. J. Histohem. Cytochem. 1974;27:1148-1162.

29. Meurs, E., Rougeot, C., Svab, J., Laurent, A. G., Hovanessian, A. G., Robert, N., Gruest, J., Montagnier, L.

and Dray, F. Use of an anti-human leukocyte interferon monoclonal antibody for the purification and radioimmunoassay of human alpha interferon. Infection and Immunity 1982;37:919-926.

30. Nagata, S., Mantei, N. and Weismann, C. Not less than eight distinct chromosomal genes for human interferon; structure of the gene for interferon-α. Nature (London) 1980;287:401-408.

31. Allen, G. and Fantes, K. H. A family of structural genes for human lymphoblastoid (leukocyte-type) interferon. Nature (London) 1980;287:408-411.

32. Goeddel, V. H., Leung, D. W., Dull, T. H., Gross, M., Lawn, R. M., Maccandlis, R., Seeburg, P. H., Ullrich, A., Yelverton, E. and Gray, P. G. The structure of eight distinct cloned human leukocyte interferon cDNAs. Nature (London) 1981;290:20-26.

33. Pestka, S. The purification and manufacture of human interferons. Sci. Am. 1983;249(2):28-35.