Brief Definitive Report

IMMUNE HUMAN LYMPHOCYTES PRODUCE AN ACID-LABILE α-INTERFERON

By F. R. BALKWILL, D. B. GRIFFIN, H. A. BAND, and P. C. L. BEVERLEY

From the Interferon Laboratory Imperial Cancer Research Fund (ICRF), Lincoln's Inn Fields, London WC2A 3PX; and the ICRF Human Tumour Immunology Group, School of Medicine, University College London, London WC1E 6JJ

Three types of human interferon (Hu IFN) have to date been characterized in terms of physicochemical properties and serology (1). Hu IFN-α and -β are mainly produced by leukocytes and fibroblasts, respectively, and are pH 2 stable but antigenically distinct (2, 3). Hu IFN-γ, produced by lymphocytes (4, 5) and T cell clones (6, 7) during mitogenic or specific antigenic stimulation, is pH 2 labile and shows no antigenic cross-reactivity with α or β (5). There are also differences in cross species reactivity between the IFN types: α-IFN is active not only in homologous cells but in other mammalian species, whereas γ-IFN is more strictly species specific (8, 9). However, we have found that when lymphocytes from individuals who have recently received influenza vaccine are stimulated in vitro with this virus, a novel IFN is produced that is pH 2 labile, but is neutralized by an antiserum to α-IFN and has activity on heterologous cells. This pH 2-labile α-IFN resembles an IFN that has been found in the serum of patients with systemic lupus erythematosus (SLE) (10).

Materials and Methods

Vaccination of Volunteers. Volunteers were vaccinated intramuscularly with MFV Ject (Institute Merieux, Lyon, France) containing a mixture of 400 IU of the following influenza viruses: A/Tex/77 (H_3N_2), A/USSR/92/77 (H_1N_1), and B/HK/8/73.

Cell Separation and Culture. Peripheral blood mononuclear cells (PBM) were separated from heparinized venous blood samples by centrifugation over Ficoll-Hypaque. 10^7 PBM were cultured at 10^6/ml in upright plastic flasks (3013; Falcon Labware, Oxnard, CA) in bicarbonate-buffered RPM1 1640 medium with added 10 mM Hepes buffer and 10% AB human serum. To obtain optimal stimulation of vaccinated volunteers, PBM were stimulated with a mixture of equal proportions of sucrose density gradient-purified influenza viruses A/JAP(H_2N_1), A/X31(H_3N_2), and A/BRAZIL (H_1N_1) at a final concentration for the mixture of 1.2 μg/ml. After 4 and 6 d in vitro stimulation, supernatants were sampled and aliquots were stored at −70°C until assayed. After sampling the volume of the cultures was made up by addition of fresh medium sampling.

Interferon Assays. Interferon was measured as the reduction of viral (Semliki Forest virus) RNA synthesis in WISH cells (Flow Laboratories, Irvine, United Kingdom [UK]), MDBK cells (Flow Laboratories) or V_3 monkey kidney cells (11), and in each experiment IFN activity in supernatants was compared with a laboratory standard calibrated against British Reference Standard 69/19 (National Institute for Biological Standards and Controls London, UK).

Characterization of IFN Activity in Supernatants. The antiserum to α-IFN was prepared by Dr. K. H. Fantes, Wellcome Research Laboratories, Beckenham, UK. A steer was repeatedly immunized with purified Hu IFN-α prepared in Namalwa cells with and without Freund's complete adjuvant. 1 ml of this antiserum neutralized 10^6 IU of HU IFN-α. IFN titers in an

aliquot of supernatant were determined; for neutralization studies the antiserum was added in the appropriate quantity to neutralize this IFN and incubated for 4 h at room temperature before assaying. Supernatants were left at pH 7 or acidified to pH 2 for 20 h at 4°C.

α-IFN Controls. Purified Hu IFN-α derived from Namalwa cells induced by Sendai virus was used as a control. This IFN is known to contain at least eight subtypes of α-IFN (12) and had a specific activity of 2.8×10^8 U/mg protein.

Results

PBM were separated from peripheral blood samples taken at intervals after vaccination from a normal volunteer and were cultured in vitro with or without influenza virus. In all cultures with the virus, blastogenic transformation and proliferation of lymphocytes occurred. Typically, the ^{125}Iododeoxyuridine incorporation in unstimulated cultures after 6 d in vitro was <100 cpm and in influenza-stimulated cultures was >3,000 cpm/10^5 cells. Table I shows the characterization of the IFN present in samples taken at the 4th or 6th d of in vitro culture of PBM with influenza virus. The PBM cultures from four volunteers were set up 7 d after vaccination. No IFN was produced in unstimulated cultures.

As shown in Table I, the IFN produced in the influenza-stimulated cultures was in all cases strongly inactivated by pH 2 treatment for 20 h but was also neutralized by an antiserum to Hu IFN-α. However, PBM cultures set up at the same time and stimulated with the mitogen concanavalin A (Con A), an inducer of γ-IFN, produced a pH 2-labile IFN that was not significantly neutralized by the α-IFN antibody. Throughout this series of experiments, PBM from donor GH failed to produce high levels of IFN when stimulated with Con A. Controls of α-IFN, induced by Sendai virus in Namalwa cells, Hu IFN-αN, and known to contain at least eight subtypes of α-IFN (12), were set up with each experiment and showed no inactivation with the pH 2 treatment, but total neutralization with the α antiserum (Table I).

Further experiments showed us that this acid-labile α-IFN was the predominant IFN produced in influenza-stimulated cultures from the four vaccinated individuals set up at various times after vaccination. So far we have examined cultures up to 49

TABLE I

IFN Activity Produced by Mitogen or Antigen Stimulation 6–7 Days Postvaccination

Volunteer	Stimulus	Treatment with			
		pH 7 20 h	pH 2 20 h	Control calf serum	Anti-α serum
		U/ml			
PB	Flu	1,450	66	1,750	22
BA	Con A	125	<8	560	450
BA	Flu	4,000	<8	4,500	540
MY	Con A	428	<8	350	300
MY	Flu	1,345	30	1,658	440
GH	Con A	6	0	3	2
GH	Flu	1,715	27	3,517	340
α controls		603	540	553	<8
		580	600	580	<8

The IFN activity in the supernatants was assayed on WISH cells as described in Materials and Methods. Supernatants from cultures of PBM from PB were harvested after 4 d of in vitro culture, and from BA, MY, and GH after 6 d. α-IFN controls consisted of highly purified Hu IFN-α as described in Materials and Methods.

d postvaccination. A summary of all the data obtained in this experimental series is shown in Table II in terms of mean percentage inactivation by pH 2 or neutralization by anti-α treatment. It is clear that influenza-induced IFN is strongly neutralized by the anti-α serum (mean neutralization, 77%), whereas Con A was not (mean neutralization, 21%), but both activities are pH 2 labile (95 and 97%, respectively). However, the α antiserum did cause a slight neutralization of the Con A-induced IFN activity; this may reflect experimental error, low-level cross-reactivity of this antibody for Hu IFN γ, or the fact that Con A induced a small amount of acid labile α-IFN as well as γ-IFN. The variability seen in the neutralization of the influenza supernatants by the α antibody may reflect a lower affinity of the antibody for this IFN, or alternatively, variable amounts of pH 2-labile γ-IFN may be produced during this in vitro immune response.

The pH 2-labile IFN produced by PBM stimulated with influenza antigen in vitro also differed from conventional γ-IFN in terms of its cross-species specificity (Table III). This IFN had greater activity on monkey and bovine cells than the IFN from Con A-induced supernatants, although it did not show the same cross-reactivity as

TABLE II

Summary of IFN Characterization

Type of sample	Number of samples tested	Mean percent reduction of titer ± SD after treatment with	
		Antiserum to α-IFN	pH 2
Con A-stimulated PBM supernatant	15	21 ±21*	97 ± 3
Influenza virus-stimulated PBM supernatant	20	77 ± 18*	95 ± 3
α control	9	95 ± 8	11 ± 13

* The difference between these two groups was found to be highly significant by the Wilcoxon two-sample rank sum test ($P < 0.0001$).

TABLE III

Cross-species Reactivity of Influenza and Con A-Induced IFN Activity

Volunteer	Stimulus in vitro	Time after vaccination	IFN activity			IFN activity on human cells after 20 h pH 2
			Human (WISH)	Monkey (V_3)	Bovine (MDBK)	
		d		*U/ml*		*U/ml*
BA	Con A	24	896	65	47	40
BA	Con A	7	1,383	28	<10	42
PB	Con A	39	3,083	85	<10	85
GH	Con A	7	98	<10	<10	23
MY	Con A	7	1,240	35	<10	5
BA	Flu	24	3,850	843	323	142
GH	Flu	7	1,870	470	160	27
MY	Flu	7	820	190	143	32
PB	Flu	39	1,463	860	650	122
BA	Flu	7	5,750	570	193	<8
α controls			909	1,000	4,230	ND*
			200	270	740	ND

Interferon activity in the supernatants was assayed on WISH, V_3, and MDBK cells in simultaneous assays.

* Not determined.

conventional α-IFN. Hu IFNα-N had equal activity on human and monkey cells and three- to fourfold more activity on bovine cells, as shown in Table III. We also investigated the heat lability of the different IFN activities. Con A- and influenza-stimulated PBM supernatants were almost completely inactivated by a 15-min incubation at 56°C (mean inactivation 87 and 90%, respectively) whereas Hu IFN-αN was not significantly inactivated (mean inactivation, 17%).

Discussion

The unusual IFN activity described in this paper probably represents an as yet uncharacterized subtype of α-IFN. There are 10 or more genes coding for different α-IFN (13), the products of which are not all clearly defined in terms of physicochemical properties. Acid-stable α-IFN is produced by leukocytes challenged with a variety of viral and nonviral stimuli (1, 8, 14, 15) and in vitro production of acid-labile IFN has been briefly mentioned in two previous studies (9, 16), but no detailed characterization has been done. In vivo acid-labile α-IFN has been described in the sera of mice infected with murine cytomegalovirus (17, and Dr. Jane Allen, personal communication) and in patients with SLE (10).

We do not know what subtype of blood mononuclear cell produces this acid-labile α-IFN. Ennis and Meager (18) have reported the production of Hu IFN-γ in an in vitro immune response to influenza virus in vaccinated individuals, but there are significant differences between their system and ours. In their experiments lymphocytes were stimulated with influenza virus in such a way as to generate cytotoxic T cells (19), whereas our cultures are known to produce helper T cells (20, 21). It will be of interest to see whether the production of different IFN types is related to different effector T cell subtypes; such experiments are currently underway.

Summary

We have described in this paper a novel human interferon (IFN) with antigenic and cross-species reactivity of α-IFN and physicochemical properties of γ-IFN. This IFN is produced by normal peripheral blood mononuclear cells during an immune response but has also been associated with autoimmune disease (10). The system described here will be useful in elucidating the biological significance and cell of origin of this IFN.

We thank Dr. K. H. Fantes, Wellcome Research Laboratories, UK, for the antiserum to Hu IFN-α, and Dr. J. Skehel, National Institute for Medical Research, UK, for the influenza virus preparations.

References

1. Stewart, W. E., II, J. E. Blalock, D. C. Burke, C. Chang, J. K. Dunnick, E. Falcoff, R. M. Friedman, G. J. Galasso, W. K. Joklik, J. T. Vikek, J. S. Youngner, and K. C. Zoon. 1980. Interferon nomenclature. *Nature (Lond.)*. **286:**110.
2. Levy-Koenig, R. E., M. J. Mundy, and K. Paucker. 1970. Immunology of interferons. I. Immune response to protective and nonprotective interferons. *J. Immunol.* **104:**785.
3. Berg, K., C. A. Ogburn, K. Paucker, K. E. Mogensen, and K. Cantell. 1975. Affinity chromatography of human leukocyte and diploid cell interferons on Sepharose-bound antibodies. *J. Immunol.* **114:**640.
4. Wheelock, E. F. 1965. Interferon-like virus-inhibitor induced in human leukocytes by phytohemagglutinin. *Science (Wash. D.C.)*. **149:**310.

5. Epstein, L. 1981. Interferon-gamma: is it really different from the other interferons? *Interferon.* **3:**13.
6. Matsuyama, M., K. Sugamura, Y. Kawade, and Y. Hinuma. 1982. Production of immune interferon by human cytotoxic T cell clones. *J. Immunol.* **129:**450.
7. McKimm-Breschkin, J. L., P. L. Mottram, W. R. Thomas, and J. F. A. P. Miller. 1982. Antigen-specific production of immune interferon by T cell lines. *J. Exp. Med.* **155:**1204.
8. Stewart, W. E. II. 1979. The Interferon System. Springer Verlag New York, Inc. 414 pp.
9. Wiranowska-Stewart, M., and W. E. Stewart II. 1981. Determination of human leukocyte populations involved in production of interferons alpha and gamma. *J. Interferon Res.* **1:**233.
10. Preble, O. T., R. J. Black, R. M. Friedman, J. H. Klippel, and J. Vilcek. 1982. Systemic lupus erythematosus: presence in human serum an unusual acid-labile leukocyte interferon. *Science (Wash. D.C.).* **216:**429.
11. Christofinis, J. G. 1970. Biological characteristics of a cell line GL-V_3 derived from the kidney of a vervet monkey *Ceropithecus aethiops. J. Med. Microbiol.* **3:**251.
12. Allen, G., K. H. Fantes, D. C. Burke, and J. Morser. 1982. Analysis and purification of human lymphoblastoid (Namalwa) interferon using a monoclonal antibody. *J. Gen. Virol.* **63:**207.
13. Weissman, C. 1981. Cloning of interferon and other mistakes. *Interferon.* **3:**101.
14. Trichieri, G., D. Santoli, and B. B. Knowles. 1977. Tumour cell lines induce interferon in human lymphocytes. *Nature (Lond.)* **270:**611.
15. Wiranowska-Stewart, M. 1981. Heterogeneity of human gamma interferon preparations: evidence for presence of alpha interferon. *J. Interferon Res.* **1:**315.
16. Epstein, L. B., D. Goldblat, K. Yu, J. H. Dean, R. B. Herberman, J. J. Oppenheim, R. E. Rocklin, and M. Sugiyama. 1980. Mediator induction in human mononuclear cells by strains of *Corynebacterium parvam. In* Biochemical Characterization of Lymphokines. A. De Weck, F. Kristensen, and P. Landy, editors. Academic Press, Inc., New York. 353.
17. Grundy, J. E., J. Trapman, J. E. Allan, G. R. Shellam, and C. J. M. Melief. 1983. Evidence for a protective role of interferon in resistance to murine cytomegalovirus and its control by non-H-2-linked genes. *Infect. Immun.* In press.
18. Ennis, F. A., and Meager, A. 1981. Immune interferon produced to high levels by antigenic stimulation of human lymphocytes with influenza virus. *J. Exp. Med.* **154:**1279.
19. McMichael, A. J., A. Ting, H. J. Zweerink, and B. A. Askonas. 1977. HLA restriction of cell-mediated lysis of influenza virus-infected human cells. *Nature (Lond.).* **270:**524.
20. Fischer, A., P. C. L. Beverley, and M. Feldman. 1981. Long term human T-helper lines producing specific helper factor reactive to influenza virus. *Nature (Lond.).* **294:**166.
21. Callard, R. E. 1979. Specific *in vitro* antibody response to influenza virus by human blood lymphocytes. *Nature (Lond.).* **282:**734.