IMMUNE HUMAN LYMPHOCYTES PRODUCE AN ACID-LABILE \( \alpha \)-INTERFERON

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Three types of human interferon (Hu IFN) have to date been characterized in terms of physicochemical properties and serology (1). Hu IFN-\( \alpha \) and -\( \beta \) are mainly produced by leukocytes and fibroblasts, respectively, and are pH 2 stable but antigenically distinct (2, 3). Hu IFN-\( \gamma \), 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 \( \alpha \) or \( \beta \) (3). There are also differences in cross species reactivity between the IFN types: \( \alpha \)-IFN is active not only in homologous cells but in other mammalian species, whereas \( \gamma \)-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 \( \alpha \)-IFN and has activity on heterologous cells. This pH 2-labile \( \alpha \)-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 (H1N2), A/USSR/92/77 (H3N1), 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 10^9/ml in upright plastic flasks (3013; Falcon Labware, Oxnard, CA) in bicarbonate-buffered RPMI 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(H2N2), A/X31(H3N2), and A/BRAZIL (H1N1) at a final concentration for the mixture of 1.2 \( \mu \)g/ml. After 4 and 6 d in vitro stimulation, supernatants were sampled and aliquots were stored at \(-70^{\circ}\)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\( _{5} \) 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 \( \alpha \)-IFN was prepared by Dr. K. H. Fantes, Wellcome Research Laboratories, Beckenham, UK. A steer was repeatedly immunized with purified Hu IFN-\( \alpha \) prepared in Namalwa cells with and without Freund's complete adjuvant. 1 ml of this antiserum neutralized 10^6 IU of HU IFN-\( \alpha \). 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 × 10⁸ 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 prolif-
eration of lymphocytes occurred. Typically, the ¹²⁵Iododeoxyuridine incorporation in
unstimulated cultures after 6 d in vitro was <100 cpm and in influenza-stimulated
cultures was >3,000 cpm/10⁶ 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

| Volunteer | Stimulus | pH 7 20 h | pH 2 20 h | Control calf serum | Anti-α serum |
|-----------|----------|-----------|-----------|-------------------|--------------|
| 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    | 429       | <8        | 950               | 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       | 533               | <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.
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**

| 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 ± 2*                                                  | 97 ± 3             |
| Influenza virus-stimulated PBM super-| 20                       | 77 ± 18*                                                 | 95 ± 3             |
| natant                               |                          |                                                          |                    |
| a 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**

| Volunteer | Stimulus in vitro | Time after vaccination | IFN activity | IFN activity on human cells after 20 h pH 2 |
|-----------|-------------------|------------------------|--------------|--------------------------------------------|
|           |                   |                        | Human (WISH) | Monkey (V₃) | 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                     | 3,650        | 843          | 323          | 142|
| GH        | Flu               | 7                      | 1,870        | 470          | 169          | 27 |
| MY        | Flu               | 7                      | 820          | 190          | 143          | 32 |
| PB        | Flu               | 39                     | 1,463        | 860          | 650          | 122|
| BA        | Flu               | 7                      | 5,750        | 570          | 193          | <8 |
| a controls|                  |                        | 909          | 1,000        | 4,230        | ND*|
|           |                   |                        | 200          | 270          | 740          | ND |

Interferon activity in the supernatants was assayed on WISH, V₃, and MDBK cells in simultaneous assays.

* Not determined.
conventional \( \alpha \)-IFN. Hu IFN\( \alpha \)-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-\( \alpha \)N was not significantly inactivated (mean inactivation, 17%).

**Discussion**

The unusual IFN activity described in this paper probably represents an as yet uncharacterized subtype of \( \alpha \)-IFN. There are 10 or more genes coding for different \( \alpha \)-IFN (13), the products of which are not all clearly defined in terms of physicochemical properties. Acid-stable \( \alpha \)-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 \( \alpha \)-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 \( \alpha \)-IFN. Ennis and Meager (18) have reported the production of Hu IFN-\( \gamma \) 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 \( \alpha \)-IFN and physicochemical properties of \( \gamma \)-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.

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