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Interferon with Novel Characteristics Produced by Human Mononuclear Leukocytes

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Treatment of human peripheral blood mononuclear leukocytes with phytohaemmaglutinin and the tumour promoter teleocidin, results in the production of large amounts of interferon-γ and significant amounts of a novel interferon-like substance which we tentatively class as interferon-δ. This novel interferon type possesses all the important characteristics of classical interferon but, of various cell types tested, has antiviral activity only in trisomy - 21 human fibroblasts. It differs decisively from previously identified interferon types in its antigenic, biological and physicochemical properties.

Human IFNs are classified as α, β or γ on the basis of their antigenicity. They differ also in their physicochemical properties and in the base sequences of their genes. There are several different but closely related IFN-α subtypes, coded for by separate genes (2-5), but probably one only of IFN-β (6) and IFN-γ (7,8). There have been reports of further heterogeneity of IFNs (9) but no clear indication of IFN antigenic types other than α, β or γ. Here we report that human PBML treated with the mitogen PHA and the tumour promoter teleocidin (10-12) produce an IFN type which differs from IFN-α, -β or -γ in physico-chemical properties. Since this IFN species is clearly antigenically distinct from IFN-α, -β, or -γ we class it as IFN-δ.

MATERIALS AND METHODS

Preparation of PBML and induction of IFN. Human PBML were isolated by centrifugation of citrate treated buffy coats (provided by the U.K. West Midlands Blood Transfusion Centre) on Ficoll-Hypaque gradients. Interface

Abbreviations
IFN: interferon; PBML: peripheral blood mononuclear leukocytes; PHA: phytohaemagglutinin.

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cells were washed and resuspended to 1-2 x 10^6 cells/ml in RPMI 1640 medium buffered with 0.0125M morpholino propane sulphonate and 0.01M bicarbonate supplemented with 10% foetal bovine serum. Interferon was induced by incubating the PBMLs with PHA (1% Gibco) and teleocidin (0.01 $\mu g\ ml^{-1}$; very generously provided by Dr. H. Fujiki, National Cancer Institute, Tokyo, Japan) for 2-4 days at 37°C in a 5% CO_2 humidified atmosphere. Teleocidin increases yields of IFN from mitogen stimulated PBML by about 10-fold (Wilkinson and Morris, MS in preparation) as do other tumour promoters, the chemically unrelated phorbol esters (13).

Assay of IFN and IFN neutralization assays. IFN assay was carried out using the inhibition of nucleic acid synthesis method (14) using Semliki Forest Virus as challenge in a variety of cell types (trisomic 21 human fibroblasts GM2504, GM2767; human amniotic cell line WISH; human foreskin fibroblasts HFF; mouse fibroblasts L929; bovine turbinate cells EBTr). IFN neutralization assays were carried out by incubating IFN preparations for 30' at 37°C with dilutions of anti IFN sera and then assaying residual IFN activity. Antisera to IFN used included an antiserum (K. Fantes, Wellcome Research, U.K.) to Human lymphoblastoid IFN which neutralizes IFN-α and IFN-β but not IFN-γ; two antisera to human leukocyte IFN which neutralize IFN-α only, one from the NIH (Bethesda, U.S.A.) and the other from Interferon Sciences Inc. (New Brunswick, U.S.A.); an antiserum to human fibroblast IFN (J. Vilcek, New York University Medical Centre) which neutralizes IFN-β only; and an antiserum prepared in this laboratory to IFN-γ produced by human PBML which neutralizes IFN-γ only.

Chromatography techniques. IFN preparations were chromatographed on conA sepharose (Pharmacia) modified matrex blue (provided by Dr. P. D. G. Dean, University of Liverpool, U.K.), and an antibody column prepared by conjugating the rabbit anti-IFN-γ antibody (mentioned above) to sepharose. Conditions for the use of these columns are indicated in the results. Gel filtration chromatography on ACA54 (LKB) equilibrated with PBS was used for molecular weight determination under non-denaturing conditions.

RESULTS

Titres of antiviral activity from PBML induced with PHA and telocidin were in the range $10^{3.5}$-$10^{4.5}$. Most of this was acid labile and neutralized by the anti-serum to IFN-γ; it was therefore considered to be IFN-γ. However, a significant proportion, about 10%, was stable to prolonged dialysis at pH2 and was initially considered to be contaminating IFN-α or -β. The antiviral activity in the supernatants was concentrated by ammonium sulphate precipitation (30-50% w/v cut) and then dialysed against PBS. This material was then sequentially chromatographed on columns of conA sepharose, modified matrex blue, and anti IFN-γ conjugated to sepharose, all equilibrated with PBS. Under these conditions IFN-α , -β and -γ were efficiently bound but the acid stable material present in the supernatants was not bound; all was recovered in the final void, free of IFN-γ.

Table 1 Antigenic characteristics of acid stable interferon

Antiserum	IFN titre (log_{10})			
	α	β	γ	novel
None	4.3	4.3	2.6	4.5
Calf anti-α IFN (lymphoblastoid)	2.5	4.3	2.5	4.5
Rabbit anti-β IFN (fibroblast)	4.1	2.3	2.6	4.4
Rabbit anti-γ IFN	4.5	4.3	1.3	4.5
Combined anti-α,-β,-γ	-	-	-	4.5

This acid stable antiviral activity conforms to the standard definition of an IFN. It was non-sedimentable at 100,000 G., non-dialysable, its activity remained after ribonuclease treatment (50 µg/ml RNase A, 100 U/ml RNase T1 for 1 hr at 37°C), but was lost when incubated in the presence of proteinase (10 µg/ml trypsin/chymotrypsin for 1 hr at 37°C). It exerted virus non-specific antiviral acitivity (with approximately equal activity against two unrelated viruses, Semliki Forest Virus and Encephalomyocarditis virus) by a mechanism involving de novo RNA synthesis (actinomycin D treated GM2767 fibroblasts do not respond to the antiviral effects of the acid stable IFN).

This IFN was further characterized using polyclonal antisera specific for human IFN-α, -β and -γ in neutralization assays. These assays reproducibly showed that this IFN was not neutralized by dilutions of antisera which effectively neutralize IFN-α (lymphoblastoid), -β or -γ, or by all antisera combined (Table 1). The two other antisera raised against leukocyte derived IFN-α, were also used in neutralization assays because of the possibility that this novel IFN species was a rare or modified IFN-α subtype not present in lymphoblastoid IFN. Neither antisera neutralized this IFN, nor did this IFN bind to a monoclonal antiobody column (15) specific for IFN-α (data not shown).

Further characterization was carried out using other physicochemical and biological tests (Table 2). The novel IFN was further differentiated

Table 2 Serological, physicochemical and biological properties of novel IFN

Property	IFN-α	IFN-β	IFN-γ	Novel IFN
Antigenic specificity	α	β	γ	non-α,-β,-γ
Stability				
pH = 2 (overnight)	stable	stable	labile	stable
0.1% SDS (1 h)	stable	stable	labile	stable
56°C (1 h)	stable	stable	labile	stable
Apparent molecular weight under non-denaturing conditions	20-25K	20-22K	40-45K	30-35K
Chromatographic behaviour				
ConA sepharose	binds	binds	binds	no binding
Modified matrex blue	binds	binds	binds	no binding
Biological antiviral activity on[a]				
Human trisomic 21 fibroblasts GM2767	100	100	100	100
Human foreskin fibroblasts	4	3	2	<0.01
Human WISH cells	21	15	21	<0.01
Bovine EBTr cells	40	0.2	<0.01	0.01
Murine L-929 cells	0.2	0.03	<0.01	<0.01

[a]Relative to activity on GM2767 = 100.

from IFN-γ by its stability after mild heat or SDS treatment. Its apparent molecular weight under non-denaturing conditions was 30-35 K as determined by gel filtration, distinct from other IFNs. The use of affinity chromatography materials commonly employed for IFN purification also showed this novel IFN to differ from IFN-α, -β or -γ. It was found not to bind to ConA sepharose or modified matrix blue, both of which bind the other IFNs under the conditions used (table 2).

Finally, the novel IFN was titrated on a range of cell types to determine its activities on homologous and heterologous cell types. It had easily detectable antiviral activity only on human trisomic 21 fibroblasts, the two tested being GM2767B and GM2504. It had no detectable activity on WISH cells, nor normal diploid fibroblasts (human foreskin fibroblasts), unlike IFN-α, -β and -γ, nor on the non-human cell lines EBTr (bovine) and

L929 (mouse) (table 2). This range of cell sensitivities is quite distinct from that of the other interferons.

DISCUSSION

These results, summarized in Tables 1 and 2, lead us to conclude that this IFN is a type which is distinct from the previously characterized IFNs by antigenic, physicochemical and biological criteria. Since IFNs are classified into types on the basis of their antigenic specificities (1), this IFN type should be tenatively considered as IFN-δ.

Whether this novel IFN is coded for by a separate gene or RNA transcript, or is generated by post translational modification of one of the classical IFNs has yet to be determined. It is possible that the major biological role of IFN-δ is not as an antiviral substance directly protecting cells against virus infection since it has little or no antiviral activity on diploid cells. This implies that it may not normally play a direct antiviral role in vivo. As classical IFNs have non-antiviral activities, including effects on the immune response (16,17), it may be that IFN-δ is primarily involved in some other activity and incidentally possesses some antiviral activity detectable only on cells particularly sensitive to IFN, trisomic 21 cells. To test this we are currently studying the effects of IFN-δ on mitogenisis in lectin-stimulated PBML and on natural cytotoxicity of lymphocytes. Our preliminary results indicate that partially purified IFN-δ preparations inhibit mitogenisis and stimulate natural cytotoxicity in fresh human PBML, indicating that IFN-δ probably exhibits the same range of biological activities as do other IFN-types. It has recently been shown that there are marked quantitative differences between IFN types in their biological properties (IFN-γ is much more potent as a stimulator of the expression of HLA antigens than IFN-α or IFN-β (18). It may be that IFN-δ is primarily an immunoregulatory molecule (as has been suggested for IFN-γ (19)) with only slight antiviral activity detected on certain cell types.

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