EFFECT OF BACTERIALLY PRODUCED INTERFERON-α_2 ON SYNTHESIS OF SPECIFIC PEPTIDES IN HUMAN PERIPHERAL LYMPHOCYTES

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Received 11 February 1982

1. Introduction

Human interferons (IFNs) are currently being produced in bacteria after genetic manipulation [1,2]. Since these IFNs may eventually be used in a clinical context, it is essential to determine whether their action on human cells is identical to that of conventional IFN. Although bacterially produced IFNs have been shown to have antiviral effects [2,3], other aspects of IFN action have not been fully explored.

In previous work we have shown that resting human peripheral blood T-lymphocytes, when exposed to interferon, exhibit enhanced synthesis of a set of eight specific peptides (I-peptides) [4]. This response is detectable within 6 h of IFN treatment and occurs with cells of all normal donors examined (>30 to date). Both IFN-α and IFN-β elicit the same response. The specificity and complexity of this response to conventionally induced IFNs suggests that it may be a useful indicator of the ability of IFNs of more exotic origin to produce the same spectrum of effects as the conventional forms. This communication describes the I-peptide enhancement obtained in human lymphocytes using virtually pure IFN-α_2, produced by *E. coli* carrying a plasmid containing the cloned cDNA for that IFN species. We found the IFN-α_2 was capable of enhancing synthesis of the same 8 peptides as previously reported. However, unlike conventionally induced IFN, the bacterially synthesized material did not produce this effect in all donors. This may reflect the complexity of the IFN-α's, which derive from at least 8 naturally occurring genes, so that a single component of the usual mixture may exhibit a limited range of donor or cell-type specificities.

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2. Materials and methods

2.1. *Peripheral lymphocytes*

Peripheral lymphocytes were obtained from heparinized blood of normal donors by nylon column adsorption and Ficoll-Hypaque sedimentation as in [5]. Resting cells were cultivated at 10^6/ml in RPMI-1640 medium supplemented with 10% autologous plasma, 50 U/ml penicillin, 50 μg/ml streptomycin, at 37°C in 7% CO_2 atmosphere.

2.2. *Labeling of proteins*

Portions containing 2×10^7 cells were incubated at 5×10^6/ml in RPMI-1640 lacking leucine, with 150 μCi/ml [^{3}H]leucine (Amersham, 60 Ci/mmol), for 4 h. Cells were then washed with cold phosphate-buffered (0.02 M, pH 7.4) isotonic saline and lysed by resuspension in 200 μl of 9.5 M urea, 2% Nonidet-P-40, 5% 2-mercapto-ethanol, 2% LKB ampholytes (pH 3.5–10). After vortexing for 10 s, nuclear residue was removed by centrifugation and the sample was stored at –80°C.

2.3. *Two-dimensional electrophoresis*

Samples containing $1-2 \times 10^6$ cpm were analyzed by 2-dimensional electrophoresis, as in [6]. Second dimension gels were 12% acrylamide. Gels were fixed, stained and dried as in [6] and fluorographs of labeled proteins were prepared as in [4].

2.4. *Interferon preparations*

The conventional IFN-α preparation used was lymphoblastoid IFN from Wellcome Laboratories, induced in Namalva cells by Sendai virus and partially purified [7] to a specific activity of 1.5×10^6 IU/mg protein. This preparation has been estimated to have <5% contamination with IFN-β, the remainder of the activ-

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

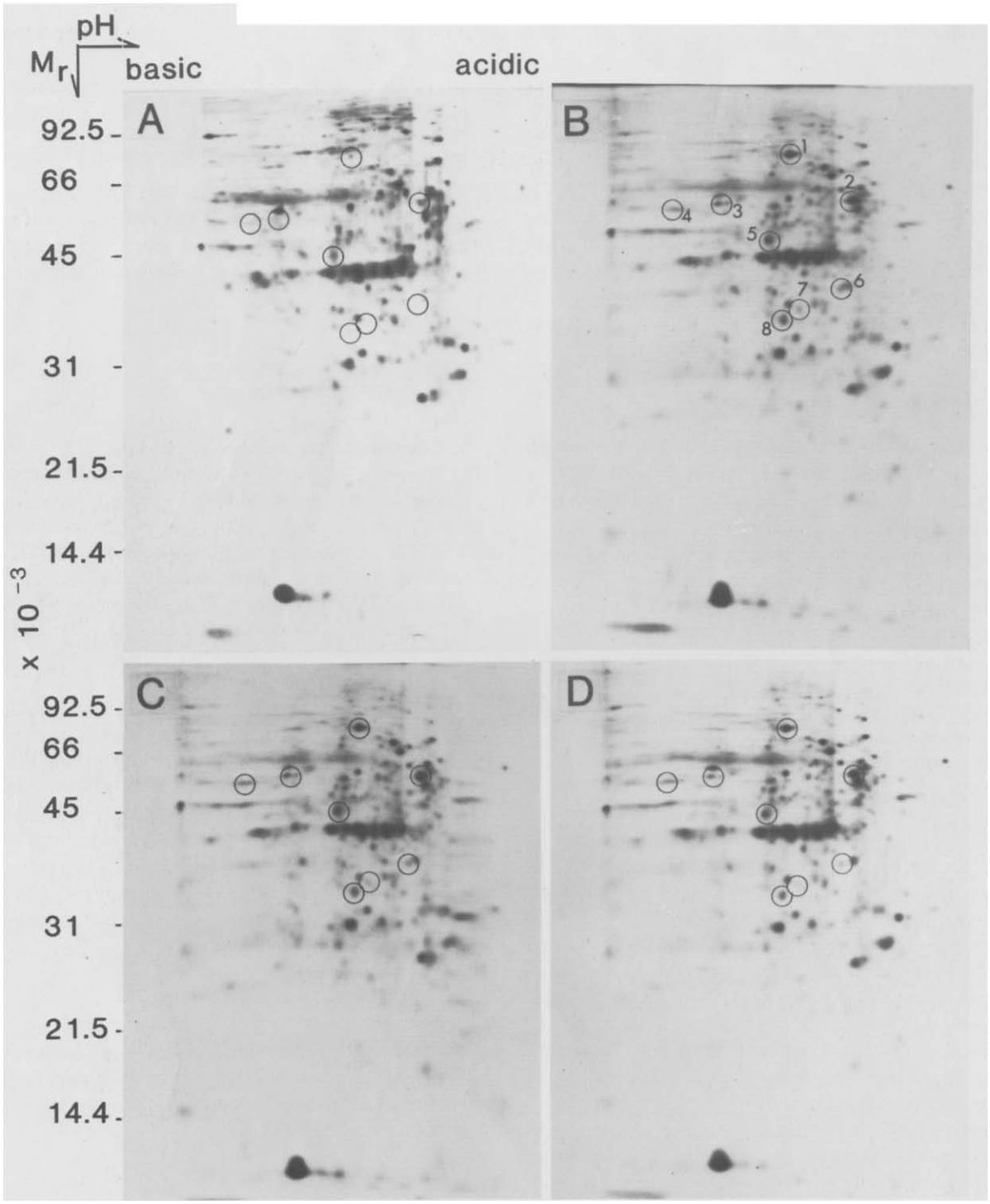

Fig.1. Effect of various IFNs on I-peptide synthesis in lymphocytes. Aliquots of resting lymphocytes containing 2×10^7 cells were incubated with 100 IU/ml of IFN for 2 h. Cells were then labeled in the presence of IFN for 4 h with [^{3}H]leucine. Cell lysates were then prepared and analyzed by 2-dimensional electrophoresis in polyacrylamide gels. First dimension (isoelectric focussing) was in 4.5% acrylamide (19:1 acrylamide:bisacrylamide), 9 M urea, 2% NP 40 and 2% (w/v) LKB ampholytes (pH 3.5–10) for 9000 V. h. Second dimension was in 12% acrylamide (36:1 acrylamide:bisacrylamide) 0.1% SDS, support buffer 0.38 M Tris–glycine (pH 8.3) for 23 h at 4.5 W, constant power. Fixation and fluorography were as in [4]. Fluorographs are shown: (A) Control; (B) IFN-β; (C) IFN-α; (D) IFN-α_2. I-peptides [1] are circled, and enumerated in (B).

ity identifiable immunologically as IFN-α (leukocyte IFN) [4]. The IFN-β preparation was fibroblast IFN, induced by poly(I):poly(C) in FS-4 fibroblasts [8] and was the generous gift of Dr E. Knight of E. I. DuPont. Its specific activity was $>10^7$ IU/mg protein. Bacterially produced IFN-α_2 was kindly provided by Drs M. Fountoulakis and C. Weissmann, Zürich, at a specific activity of 1.5×10^8 IU/mg of protein. It was produced in *E. coli* carrying a modified IFN-α_2 cDNA [9] and was purified as in [3]. A polyacrylamide gel electrophoretic analysis, provided with the sample, showed this material to have a single major band and several faint minor bands.

3. Results

Freshly prepared lymphocytes from unselected normal donors were exposed to various IFN preparations for 6 h and the proteins synthesized during the final 4 h of incubation were analyzed by 2-dimensional electrophoresis.

Treatment with conventional IFN-α and IFN-β at 100 U/ml produced enhanced synthesis of the same set of I-peptides as report in [4] (fig.1A–C).

Of the set of I-peptides usually seen, peptides I-6 and I-7 are the most difficult to detect and document photographically. However, enhanced synthesis of these peptides was detected on the original fluorographs in all IFN-treated samples. We have shown that such visual judgements can be made reliably when labeling of a peptide has increased 2-fold or more relative to its neighbor peptides [4].

Exposure to bacterially produced IFN-α_2 at the same concentration as conventional IFNs (100 U/ml), produced an identical enhancement of I-peptide synthesis (fig.1D). Apart from the I-peptide response, no other alterations, produced specifically by IFN-α_2, were detected. Thus IFN-α_2 produced by bacteria can elicit a complex biochemical response from normal human cells, freshly isolated from the body, indistinguishable from their response to conventional IFNs.

Repetition of this experiment, however, revealed that the lymphocytes of some donors responded only minimally to IFN-α_2, in terms of I-peptide synthesis, while showing the usual prominent response to IFN-β.

Thus, the lymphocytes of the donor analyzed in fig.2 revealed a barely detectable response to IFN-α_2, most easily detected by comparison of peptide I-1 with the control preparation (fig.2A,B). The other I-peptides were also very slightly enhanced, although photographic reproduction renders documentation difficult. These cells responded strongly to IFN-β, however (fig.2C).

This donor's 2-dimensional protein map, interestingly, presents an apparent genetic variant, evident as a relatively heavily-labeled peptide which obscures protein I-6 in both control and IFN-treated samples.

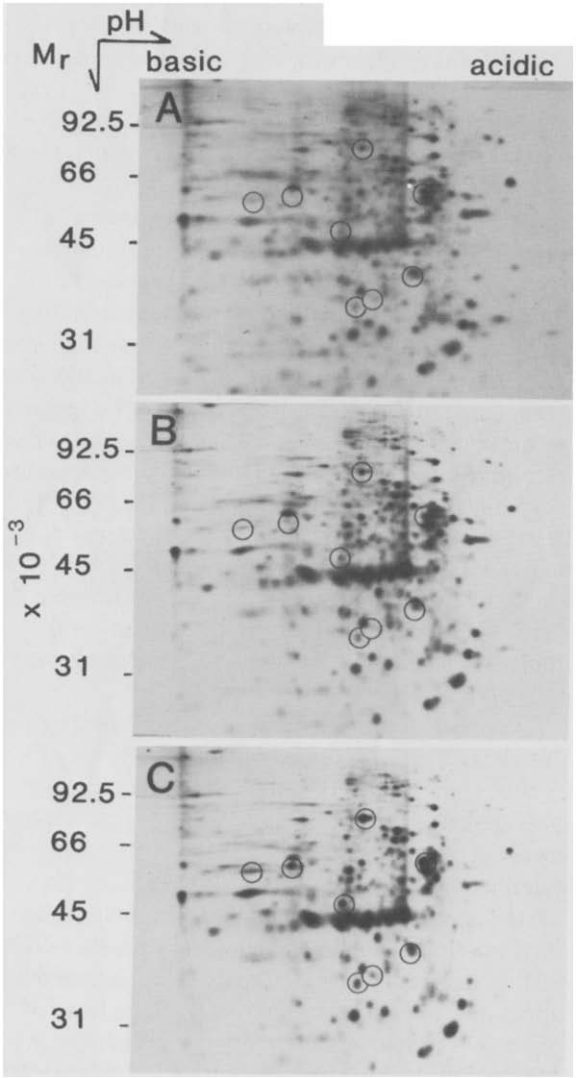

Fig.2. I-peptide synthesis in a donor showing reduced responsiveness to IFN-α_2. Conditions as in fig.1: (A) Control; (B) IFN-α_2; (C) IFN-β. In this donor, peptide I-6 was obscured by another more heavily labeled peptide which migrates to nearly the same position.

4. Discussion

The IFN concentration used in this study (100 IU/ml), has never previously failed to elicit a maximal I-peptide response, using conventional IFN-α or IFN-β. Therefore, we conclude that IFN-α_2 produced in bacteria, although capable of eliciting a typical I-peptide response, shows a degree of selectivity among lymphocytes of different normal donors not found with conventional IFNs. Such selectivity is not entirely unexpected. At least 8 IFN-α genes, each producing a slightly different product, have been identified [10,11], while only one or two IFN-β genes have been detected [12]. Different degrees of cross-species viral protection have been reported for two of the IFN-α's [9], a finding which may have the same origin as the selectivity seen here.

When IFN-α_2 induced an I-peptide response, all eight peptides were enhanced as a group; the reduced response similarly encompassed the complete set. This suggests a coordinate response to the initiating stimulus which is, presumably, the binding of a receptor site. Differences in response to different IFN-α's, then, may reflect genetic or functional heterogeneity of the receptor. The various IFN-α's, as a group, may recognize the entire receptor range, with each member adapted to a segment of that range. IFN-β, apparently, recognizes the entire receptor range, since no donor in our experience has failed to respond. Further experience with other members of the IFN-α complex, as they become available in sufficient quantity and purity, should clarify the question of receptor range recognition.

Previously, bacterially produced α-IFNs have shown antiviral activity [2,3] of comparable efficiency to that of conventional IFN-α [3]. The present report provides evidence that the complex biological response involved in controlling the synthesis of 8 specific peptides in human lymphocytes is also equivalent for bacterially produced and conventional IFNs. This increases the likelihood that bacterially produced IFNs will be an acceptable and potentially more available alternative to conventional IFNs. Such evidence of equivalent physiological activity is especially important in view of the recent demonstration that conventional IFN-α may lack some 10 COOH-terminal amino acids predicted from the sequence analysis of DNA clones used to direct IFN synthesis by bacteria [13].

The results reported here suggest the usefulness of this approach in screening potential recipients of IFN for clinical purposes, to determine responsiveness and to estimate sensitivity by using limiting IFN concentrations. The numbers of lymphocytes needed for such studies can be obtained from patients without risk and the amounts of IFN needed are small (<2000 units/test). Since the lymphocytes are used immediately, without exposure to extended in vitro cultivation or to heterologous materials (autologous plasma, or serum-free conditions may be used), the most direct approach to individual cellular response is afforded without any patient risk.

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