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Immunology

A species of human α interferon that lacks the ability to boost human natural killer activity

(cytolysis/recombinant)

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ABSTRACT **Most species of recombinant leukocyte interferons (IFN-αA, -αB, -αC, -αD, -αF, -αI, and -αK) were capable of boosting human natural killer (NK) activity after a 2-hr treatment of cells at a concentration of 1–80 units/ml. In contrast, recombinant human IFN-αJ was found to be incapable of augmenting NK activity after exposure of cells for 2 hr to concentrations as high as 10,000 units/ml. This inability of IFN-αJ to boost NK activity was not complete because, after exposure of cells to a high concentration of IFN-αJ (10,000 units/ml) for 18 hr, boosting of cytolysis was observed. IFN-αJ appeared to interact with receptors for IFN on NK cells since it was found to interfere with the boosting of NK activity by other species of IFN-α. In contrast to its deficient ability to augment NK activity, IFN-αJ has potent antiviral and antiproliferative activities. Such extensive dissociation of these biological activities has not been observed previously with any other natural or recombinant IFN species. Thus, this IFN species may be useful for evaluating the relative importance of various biological activities on the therapeutic effects of IFN, for understanding structure–function relationships, and for determining the biochemical pathways related to the various biological effects of IFN.**

Human leukocyte interferon (IFN-α) is a family of individual species differing in amino acid sequences (1–3). Several recombinant IFN-α molecules and hybrid IFNs have been expressed in *Escherichia coli*, purified to homogeneity, and characterized (1, 4–6). These recombinant and hybrid IFNs as well as natural IFNs vary considerably in their patterns of antiviral activity on human, bovine, mouse, feline, and rat cells. IFNs also have been shown to modify a wide variety of biological responses, and previous studies (1, 7–11) have indicated substantial quantitative differences among the natural and recombinant IFN species in their relative potency in exerting antiviral, antiproliferative, and immunomodulating activities. Previous reports (9, 12–16) have demonstrated that natural killer (NK) cell activity can be significantly augmented by natural and recombinant IFN-α species. However, each of the species previously tested exhibited an ability to mediate all of these effects. In this report we describe the dissociation of the antiviral activities of IFN-αJ from its ability to stimulate NK cells.

MATERIALS AND METHODS

Recombinant human IFN-αA, -αB, -αC, -αD, -αF, -αI, -αJ, and -αK were prepared by modification of methods previously described (5, 6). Unless otherwise noted, the specific activity of the preparations on MDBK cells was about 1×10^8 units/mg or greater with respect to the human IFN-α reference standard (17). Preparations with specific activities $\geq 1 \times 10^8$ units/mg were at least 90% homogeneous as determined by $NaDodSO_4$/polyacrylamide gel electrophoresis (5, 6). IFN-αB represented about 75% of the total protein. IFN-αF was a crude bacterial extract (4). All IFN titers were determined with respect to the human IFN-α reference standards G-023-901-527 and MRC 69/19 obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD) and the International Laboratory for Biological Standards, National Institute for Biological Standards and Control (Holly Hill, Hempstead, London), respectively. Effector cells were low-density fractions from Percoll density gradient-separated peripheral blood mononuclear cells from normal human adult donors. These cells contained high percentages of large granular lymphocytes (LGL), which account for human NK activity (18). K562, a cell line derived from a patient with chronic myelogenous leukemia in blast crisis, was used as the target cell for NK activity, which was measured in a 4-hr ^{51}Cr-release assay (9). To assess the effects of IFN, effector cells were incubated with various concentrations of IFN in RPMI 1640 medium containing 5% pooled human AB serum in a final volume of 1 ml for 2 hr at 37°C, washed, and then tested for cytotoxic activity. Data are expressed as lytic units per 10^7 cells, with one lytic unit defined

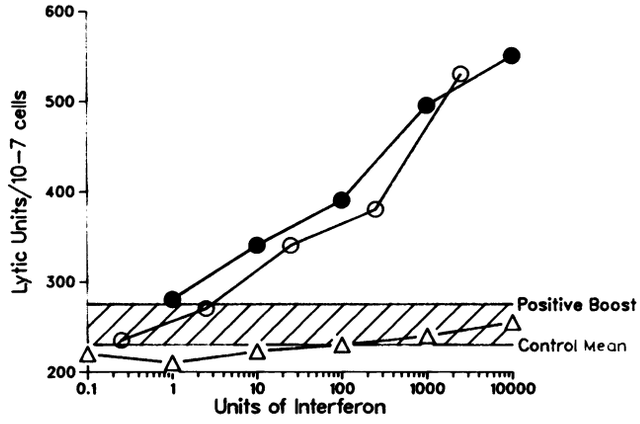

FIG. 1. Effect of various concentrations, expressed in antiviral units, of IFN-αA (○), IFN-αB (●), and IFN-αJ (△) on boosting NK activity of purified human LGL. Cells were preincubated with the designated IFN for 2 hr at 37°C, washed, and tested in a 4-hr ^{51}Cr-release assay. Data are expressed as lytic units per 10^7 cells. A positive boost is defined as a significant increase, $P < 0.05$ or ≥1.6 standard deviations above the mean of triplicate controls, incubated under the same conditions without IFN.

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Abbreviations: IFN, interferon; IFN-α, leukocyte IFN; NK, natural killer; LGL, large granular lymphocytes.

Table 1. Effect of 18-hr pretreatment with IFN-α on NK boosting

IFN species	IFN added	Lytic units per 10^7 cells
Control	—	99 ± 11.8
IFN-αA	1,000	226*
	100	213*
	10	166*
	1	123*
IFN-αJ	10,000	167*
	1,000	117
	100	96
	10	82

*Significant boosting of NK based on the mean of three controls ± 1.6 standard deviations ($P \leq 0.05$).

as the number of cells required to produce 30% specific cytotoxicity (9, 18).

RESULTS

Fig. 1 shows a representative experiment evaluating the effects of various concentrations of IFN-αA, IFN-αB, and IFN-αJ on augmentation of NK activity after 2 hr of exposure of LGL to IFN. A clear dose–response relationship was seen with IFN-αA and IFN-αB, with less than 10 units of IFN required for significant boosting of NK activity. In contrast, incubation of LGL for 2 hr with up to 10,000 units of IFN-αJ per ml did not result in significant boosting of human NK activity. After exposure of cells for 18 hr, IFN-αJ boosted NK activity, but only at concentrations greater than 1000 units/ml (Table 1). All samples were verified to have maintained their antiviral activity at the time of NK boosting by testing aliquots simultaneously in a standard IFN assay (17). Experiments (not shown) have demonstrated antiproliferative activity of IFN-αJ on Daudi cells. Fifty percent inhibition of growth was seen at 20 units/ml, a concentration at which IFN-αJ had approximately the same effect. Thus, this recombinant species was selectively unable to rapidly boost NK cytolytic activity. Even with prolonged preincubation, it was much less potent than the other IFN-α species, with about 1000- to 10,000-fold more IFN-α than IFN-αA or other species required for comparable boosting. Identical results were obtained with purified homogeneous and crude preparations of IFN-αJ.

The species of human IFN-αA, -αB, -αC, -αD, -αF, -αI, -αJ, and -αK generally showed great biological diversity (Table 2). The mean, median, and range of units of IFN activity that were required for significant augmentation of biological activity in the NK assay after a 2-hr incubation are summarized in Table 2. The rank order of potency of the IFNs in stimulating NK cells, based on the mean values from five experiments, was B ≅ A ≅ C > D > I > K > F > J. Because IFN-αJ was the only preparation that did not boost NK activity, it is particularly useful for further study of the mechanism of NK boosting by IFN.

Table 2. Ability of recombinant IFNs to augment human NK activity

IFN species	Augmenting dose*		
	Mean	Median	Range
IFN-αA	1.5	0.5	0.5–5
IFN-αB	1.1	0.1	0.01–5
IFN-αC	1.8	1.0	0.07–5
IFN-αD	7	7	5–13
IFN-αF	89.2	80	27–170
IFN-αI	10.7	8.2	1.0–29
IFN-αJ	>10,000	>10,000	>10,000†
IFN-αK	29	23	12–42

*Minimal IFN titer (antiviral units/ml) required for augmenting NK activity in a 2-hr pretreatment. Five experiments were run for each species.
†No boost at 10,000 units/ml.

Table 3. Effect of IFN-αJ on the boosting of NK activity of LGL by other IFN-α species

Treatment	IFN added,* units/ml	Lytic units per 10^7 cells
Control	—	100
IFN-αA	1000	310
IFN-αB	1000	300
IFN-αJ	1000	110
IFN-αA/IFN-αJ	1000/1000	130
IFN-aB/IFN-αJ	1000/1000	110
IFN-αJ, then IFN-αA	1000/1000	110
IFN-αJ, then IFN-αB	1000/1000	110
IFN-αA, then IFN-αJ	1000/1000	280
IFN-αB, then IFN-αJ	1000/1000	275

LGL were preincubated at 37°C for 2 hr with one or two species of IFN or, as noted, for 1 hr with one species followed by the addition of another species for 1 hr of further incubation, washed, and then tested for cytotoxic activity.
*In antiviral units.

In an attempt to determine whether the inability to boost NK with a 2-hr pretreatment with IFN-αJ was due to poor binding to NK cells or to ineffective triggering of the cytotoxic function, we examined the ability of IFN-αJ to inhibit the rapid boosting of NK activity by IFN-αA or IFN-αB. As shown in Table 3, the presence of IFN-αJ in the culture interfered with the ability of either IFN-αA or IFN-αB to augment NK cytolytic activity. In addition, exposure of effector cells with the IFN-αJ for 1 hr before addition of IFN-αA or IFN-αB also completely abrogated the augmentation of NK activity. In contrast, IFN-αJ had little or no inhibitory effects on the boosting by either IFN-αA or IFN-αB when it was added after preincubation with the other IFNs. These results indicate that IFN-αJ has the ability to interact with NK cells and to interfere with effective stimulation by other species of IFN. The apparent competitive nature of this inhibition was supported by the ability of high concentrations of IFN-αA (>2,000 units/ml) to overcome the inhibitory effects of 200 units of IFN-αJ per ml (Table 4). Although the IFN-αJ preparation was highly purified, it was important to confirm that the inhibitory effects described were in fact due to IFN and not to a minor contaminant. Therefore, we examined the preparation for its sensitivity to heat inactivation. Heating of IFN preparations at 65°C for 30 min is known to reduce or eliminate antiviral activity, and such treatment eliminated the ability of IFN-αA to boost NK activity (Table

Table 4. Inhibition of boosting of NK activity by various concentrations of IFN-αA

Preparation	Dose of IFN-αA	Dose of IFN-αJ	Lytic units per 10^7 cells
Control	—	—	295
IFN-αA	20,000	—	451*
IFN-αA	2,000	—	436*
IFN-αA	200	—	393*
IFN-αA/IFN-αJ	20,000	200	417*
IFN-αA/IFN-αJ	2,000	200	388*
IFN-αA/IFN-αJ	200	200	321
IFN-αJ	—	200	310
IFN-αA (65°C/30 min)	200	—	287
IFN-αA/IFN-αJ	200	200 (65°C/30 min)	403*

Significant boosting from control (mean of three replicate controls incubated under the same conditions without IFN) at $P \leq 0.05$.

Table 5. Differences between amino acids in IFN-αJ and other recombinant IFN-α species

IFN	Minimal concentration required for boosting NK activity*	Amino acid at position					
		10	35	40	46	116	132
IFN-αA	0.5	Gly	Asp	Gln	Asn	Ser	Lys
IFN-αB	0.1	Gly	Asp	Gln	Lys	Ser	Thr
IFN-αC	1.8	Gly	Asp	Gln	Asn	Ser	Ile
IFN-αD	7.0	Asp	Asp	Gln	Asn	Ser	Thr
IFN-αF	89.2	Gly	Asp	Gln	Asn	Ser	Thr
IFN-αH	NT†	Asn	Asp	Gln	Asn	Ser	**Met**
IFN-αI	10.7	Gly	Asp	Gln	Asn	Ser	Thr
IFN-αJ	>10,000	**Arg**	**Glu**	**Glu**	**His**	**Phe**	**Met**
IFN-αK	27.2	Gly	Asp	Gln	Asn	Ser	Thr
IFN-αL	NT†	**Arg**	Asp	Gln	Asn	Ser	Ile

*Antiviral units/ml.
†Not testable since the genes for IFN-αH and IFN-αL have not yet been expressed in *E. coli*.

4). Such treatment of IFN-αJ resulted in complete abolition of its antiviral activity and also of its ability to inhibit the augmentation of NK activity by the other interferon species (Table 4).

DISCUSSION

IFN-αJ has been shown to lack detectable ability to augment NK activity after 2 hr, although it exhibits antiviral and antiproliferative activities. Although other species of natural and recombinant IFNs have shown considerable quantitative variation in their relative potency in these three biological assays (1, 5, 10, 11), this report describes a major dissociation requiring 1000- to 10,000-fold more antiviral units of IFN and prolonged incubation to result in effects similar to the other species tested. In addition to its defective ability to stimulate NK activity, it is also possible that IFN-αJ may lack some other biological activities such as the pyrogenic and other side effects (19).

Several explanations may be considered for the deficiency of IFN-αJ to boost NK activity. (*i*) It is possible that this species of IFN might have toxic effects on NK cells. However, this seems to be ruled out by the lack of any effect of IFN-αJ on the spontaneous levels of NK activity nor on the viability of the cells. Furthermore, the boosting of NK activity by either IFN-αA or IFN-αB was not abrogated by subsequent addition of IFN-αJ. (*ii*) Inactivation of the functional properties of IFN-αJ might have occurred during the handling of this preparation. However, this possibility was ruled out by our demonstration that it retained its original level of antiviral activity. (*iii*) We considered the possibility that the IFN-αJ species might lack the ability to rapidly bind to appropriate cell surface receptors on NK cells. However, the ability of a 1-hr preincubation with IFN-αJ to completely block the augmentation of NK activity by the other species of IFN indicates that IFN-αJ can competitively interact with the same IFN receptors. Furthermore, incubation of purified NK cells for a prolonged period with high concentrations of IFN-αJ resulted in significant augmentation of NK activity. However, the efficiency of binding of IFN-αJ to NK cells must be directly examined by binding studies with radiolabeled preparations of IFN. (*iv*) Since IFN-αJ seems to bind to receptors on NK cells as well as to the cells used for the antiviral and antiproliferative assays, we postulate that IFN-αJ lacks the ability to efficiently trigger the metabolic pathway(s) required for stimulation of NK activity. Metabolic changes have been shown to be induced in cells treated with IFN, such as induction of synthesis of (2′-5′)-oligoadenylate (20) and stimulation of a protein kinase (20, 21). Preliminary results (data not shown) indicated that induction of (2′-5′)-oligoadenylate synthetase in cells treated with IFN-αJ is delayed and decreased compared to cells exposed to IFN-αA. Further studies with cells treated with IFN-αJ may help to provide some insight into which effects are related to stimulation of NK activity.

The marked dissociation of biological effects induced by IFN-αJ adds new information about the structure–function relationships of IFN. Previous studies with hybrid recombinant molecules, consisting of various portions of IFN-αA and IFN-αD, have delineated some major quantitative differences in their relative abilities to induce antiviral, antiproliferative, or NK activity (1, 5, 10, 11, 18). Introduction of three amino acids from IFN-αD into the 68–91 amino acid positions of IFN-αA resulted in a relatively greater decrease in NK-boosting potency than in its ability to exert antiviral or antiproliferative effects (10, 11). By reviewing the reported sequences of the IFN-α species (1), one may note that IFN-αJ differs from most other IFN-α species by at least 5 or 6 positions—i.e., 10, 35, 40, 46, 116, 132 (Table 5). The contribution of each of these amino acid substitutions to the defective stimulation of NK activity might be directly tested by introducing selective alterations in the sequence of IFN-α molecules.

The identification of an IFN-α species with antiviral and antiproliferative activities but deficient NK-augmenting activity also provides an approach to dissection of the relative importance of these activities for the *in vivo* antitumor effects of IFN. It would be of interest to explore the relative therapeutic efficacy of IFN-αJ in patients or in nonhuman primates with tumors shown to be responsive to IFN-αA or other species of IFN-α.

These results clearly demonstrate that mixtures of human IFN-α species exhibit properties different from the individual IFNs. In this case, the NK activity of IFN-αA and IFN-αB can be blocked by IFN-αJ. Since it appears that all of these IFNs can enter the cellular receptor, it seems that the structure of IFN-αJ permits it to trigger the mechanisms that generate antiviral and antiproliferative activity but not those for stimulating NK activity. These results are in accord with the hypothesis that the IFN receptor is a complex site that contains several independent triggers (10, 11).

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