Effect of Interferon-α1 from *E. coli* on Some Cell Functions

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Summary. Interferon-α1 from *Escherichia coli* transformed with a hybrid plasmid containing a human leukocyte complementary DNA insert, induces resistance to virus in appropriate target cells. It also shares the following properties with natural leukocyte interferon (IFN). (i) It enhances natural killing activity of human lymphocytes, (ii) it enhances antibody-dependent cell-mediated cytotoxicity, (iii) it suppresses antigen- and mitogen-induced leukocyte migration inhibition, (iv) it inhibits growth of IFN-sensitive Burkitt lymphoma cells. Since these activities are exhibited by a cloned protein species, they are due to IFN itself and not to other human proteins.

Leukocytes and fibroblasts produce interferon (IFN) in response to virus infection and to certain natural and synthetic compounds. Lymphocytes can also be induced to make IFN by polyclonal activators, specific antigens, or interaction with various cell lines. Three distinct types of IFN are distinguished by serological and biochemical criteria: IFN-α, such as the virus-induced acid-stable leukocyte or lymphoblastoid IFN's; IFN-β—for example, the acid-stable major component of IFN produced by fibroblasts; and IFN-γ, represented by the acid-labile IFN's produced by lymphocytes engaged in an immune response (*1*).

The IFN binds to specific receptors in the plasma membrane and regulates a variety of cellular functions. Evidence from many sources suggests that IFN's are also important modulators of the immune response, which they can either suppress or enhance (*2*).

The two main sources of human IFN are normal leukocytes and established cell lines of human origin, particularly fibroblasts and the Burkitt lymphoma line, Namalwa. Production of IFN from human cells is costly and cumbersome, and therefore alternative sources are being sought. Nagata *et al.* (*3*) recently cloned human leukocyte IFN complementary DNA's (cDNA's). They used them to elicit production in *Escherichia coli* of polypeptides that have human leukocyte IFN activity, as judged by biochemical and immunological evidence, and by functional tests for antiviral activity (*3*).

At least a substantial fraction of human leukocyte IFN from natural sources is believed to be glycosylated (*4, 5*). Most likely *E. coli*-derived IFN is not glycosylated, or at least not in the same way as the natural human compound. It was therefore important to test the material produced by *E. coli* for other types of activities associated with human IFN.

We have compared IFN-α1 (*E. coli*), a polypeptide related to a human leukocyte IFN species, produced by *E. coli* harboring the hybrid plasmid pBR322/SN35-AH-L6 (Fig. 1), with human leukocyte IFN preparations with regard to the following functions, previously known to be affected by human IFN: (i) enhancement of the natural killing activity of human blood lymphocytes; (ii) enhancement of antibody-dependent cell-mediated cytotoxicity (ADCC); (iii) suppression, in vitro, of antigen- and mitogen-induced delayed hypersensitivity response, as assayed by leukocyte migration inhibition (LMI); (iv) growth inhibition of the IFN-sensitive Burkitt lymphoma line, Daudi.

We also undertook to explore whether the IFN made by *E. coli*, whose molecular structure may differ from that of its naturally occuring counterpart, has the same target cell specificity as IFN from human leukocytes or lymphoblastoid cells. We have therefore tested the effect of IFN-α1 (*E. coli*) and lymphoblastoid (Namalwa) IFN on natural cytotoxicity and leukocyte and macrophage migration inhibition in both the human and mouse systems.

Enhancement of natural cytotoxicity. The cytotoxic lymphocyte population, which has a lytic effect on a number of cultured cell lines, is heterogeneous with regard to cell surface markers. The operationally defined "null" cell subset—obtained after the removal of sheep blood cell-rosetting T cells and surface immunoglobulin-positive B cells—is highly enriched in lytic activity (*6*). This subset contains a high proportion of Fc (Fc, a fragment of immunoglobulins) receptor-positive cells (*7*). T lymphocytes with low avidity red cell receptors are also active (*7, 8*). In short-term tests, leukemia lines derived from T cells are usually more sensitive than lines derived from B cells (*9*). In the latter group, B cell lines of neoplastic (lymphoma) origin are often more sensitive than lymphoblastoid lines derived from nonmalignant sources (*10*). Two lines, Daudi and Raji, derived from Burkitt lymphoma were chosen for our study. In short-term assays these two cell lines are virtually insensitive to the cytotoxicity of untreated lymphocytes (*11*), but they can be lysed by IFN-activated lymphocytes (*12*). In prolonged assays, these target cells are also lysed by nonactivated lymphocytes (*13*); under these conditions, IFN production is frequently detected during the test period.

The boosting effect of IFN is most clearly apparent when insensitive targets for natural killer cells are used, since lines that are highly sensitive to natural killing, such as K562, are readily killed by manipulated effectors, and IFN has no effect or only a minor effect (*13*). In the mouse system, we used the YAC-1 line, which is highly sensitive to natural killing.

The IFN from both Namalwa cells and IFN-α1 (*E. coli*) were efficient in activating human lymphocytes for cytotoxicity against Daudi and Raji cells. However, there was an important difference between the two IFN preparations. Namalwa IFN stimulated human but not

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mouse effector cells, while the IFN from *E. coli* enhanced the cytotoxic activity of both human and mouse effector cells (Fig. 1B). This effect was seen with lymphocytes from two different mouse strains, one with high (from CBA mice) (Fig. 1B) and one with low (from A mice) natural killing activity (not shown). The level of enhancement by IFN of lymphocyte cytotoxicity was dose-dependent in both the human and mouse systems (Fig. 2).

Enhancement of ADCC. Lymphocytes that carry Fc receptors bind to and can kill target cells coated with antibodies. The specificity of this reaction is determined entirely by the antibody; the effector cells themselves have no selective role (*14*). Treatment of lymphocytes with IFN can boost their ADCC effect, probably as a result of an increase in expression of their Fc receptors (*15*). We used an EBV-transformed (EBV, Epstein-Barr virus) lymphoblastoid cell line derived from a normal donor as the target cell. This line (GM) is resistant to both natural and IFN-induced cytotoxicity. Antibodies directed against the surface immunoglobulin render the cells sensitive to ADCC. Preliminary exposure of the effector lymphocytes to Namalwa IFN or Le IFN-α1 (*E. coli*) enhanced the ADCC reaction considerably (Fig. 3).

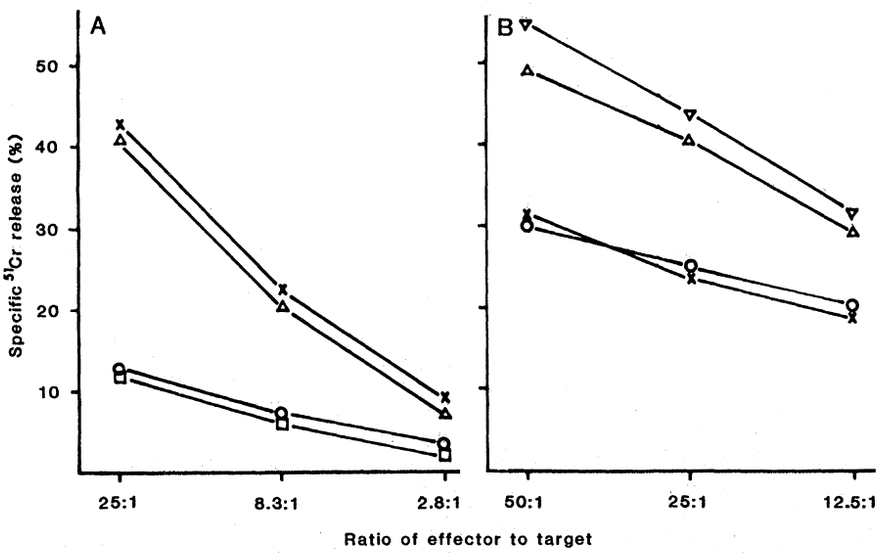


Fig. 1. Cytotoxicity of human and mouse lymphocytes induced by IFN. The IFN-α1 (*E. coli*) was extracted from *E. coli* HB101 containing plasmid Z-pBR322(Pst)/HcIF-SN35-AH-L6 (Hif-L6, for short). This plasmid is derived from Z-pBR322(Pst)/HcIF-SN35 (*3*) by insertion of a 95-bp Alu I fragment containing the lac UV5 promotor of *E. coli* (*32*). Bacteria were grown and harvested (*3*) and placed in a homogenizer with 2.5 times their weight of glass beads and once their weight of phosphate-buffered saline (PBS), for 12 minutes at 4° to 10°C. The homogenate was adjusted to 0.5*M* NaCl and 1 percent polyethylenimine (PEI), and a heavy precipitate was removed after centrifugation for 20 minutes at 8000*g*. The clear supernatant, which contained all IFN activity, was adjusted to 80 percent saturation with ammonium sulfate. After 4 hours at 0°C, the precipitate was collected by centrifugation; dissolved in the minimal volume of PBS, *p*H 7; and dialyzed for 18 hours against 100 volumes of glycine-HCl buffer (*p*H 2.2), and then for 18 hours against two 100-volume portions of PBS. A heavy precipitate was removed by centrifugation. The IFN in the supernatant was concentrated by ammonium sulfate precipitation as above and subjected to chromatography on Sephadex G-100 (*3*). The resulting preparation had a specific activity of 4200 international units per milligram of protein. A control preparation, starting from *E. coli* HB101 containing plasmid pBR322, was processed in parallel. Human leukocyte IFN (specific activity, 2.5×10^5 international units per milligram of protein) was prepared from cultured Namalwa cells, as described by Bodo (*33*). Human lymphocytes were separated from heparinized blood of healthy donors. The blood was centrifuged on Ficoll-Isopaque gradients, and the lymphocytes were collected from the interphase and depleted of macrophages by iron treatment (*34*). Mouse lymphocytes were obtained from a spleen cell suspension prepared from two CBA donors after the erythrocytes were lysed by distilled water. The lymphocytes were first incubated for 2 hours at a concentration of 10^6 cells per milliliter in RPMI 1640 medium containing 10 percent fetal calf serum (FCS) (○) (control); or 1000 international units per milliliter of partially purified IFN-α1 (*E. coli*) (△); or *E. coli* control extract, adjusted to the same protein concentration as the IFN-α1 preparation (□); or 1000 international units per milliliter of human leukocyte IFN prepared from Namalwa cells (×). Mouse lymphocytes were also incubated in the presence of 1000 international units per milliliter of mouse IFN (specific activity, 5.6×10^6 international units per milligram of protein) (Calbiochem-Behring, La Jolla, California) (▽). After incubation, the cells were washed and tested for cytotoxicity in a 4-hour ^{51}Cr release assay (*35*). (A) Human effector cells were titrated against 4×10^3 Daudi target cells. (B) Mouse effector cells were titrated against 10^4 YAC-1 target cells. Specific ^{51}Cr release was calculated as 100 × (stimulated release − spontaneous release)/(total − spontaneous release). Spontaneous release of ^{51}Cr from Daudi and YAC cells was 6 and 17 percent, respectively.

Effect on human LMI. The mobility of blood granulocytes can be measured quantitatively in vitro. It can be inhibited by a lymphocyte-derived factor (or factors) released after the interaction of lymphocytes with mitogens or antigens to which they had been pre-sensitized. The LMI test is regarded as an in vitro correlate of delayed hypersensitivity (*16*).

We found previously (*17, 19*) that IFN reduces the inhibition of the mobility of blood granulocytes by phytohemagglutinin-stimulated lymphocytes (*18, 19*) from 61 to 32 percent at an optimal concentration of 10^2 IFN units per milliliter; IFN at the same level had no detectable effect on the spontaneous migration of granulocytes in the absence of lymphocytes.

The viability of the lymphocytes was 95 percent after 24 hours of cultivation, as judged by the trypan blue exclusion test. These findings rule out the possibility that IFN had a toxic effect on the system and support the interpretation that IFN acts directly on the lymphocytes either by antagonizing the activation step, or by suppressing the production of migration inhibitory factor (*20*).

The results of one of three experiments where IFN from human lymphoblastoid cells and IFN-α1 produced by *E. coli* were tested in this system (LMI) are shown in Fig. 4. Both preparations reduced the mitogen PHA-induced human LMI to a similar extent (Fig. 4). The two IFN preparations were also equally active in the EBV antigen-specific LMI test. In this test, the effect of IFN on leukocyte migration is measured in the presence of lymphocytes from an EBV-seropositive donor challenged with the antigenic extract of the EBV-carrying P_3HR-1 cell line (*21*) (Fig. 4).

Effect on murine macrophage migration inhibition (MMI). In a reaction analogous to human LMI, the migration of mouse macrophages can be inhibited by a lymphokine designated as macrophage migration inhibitory factor (MIF). In this study, we have tested the effect of IFN on a lectin (concanavalin A, con A) and antigen-induced migration inhibition of mouse peritoneal macrophages. As shown in Fig. 5, human Namalwa IFN had no effect on the con A–induced MMI, whereas mouse IFN decreased the MMI, as expected. IFN-α1 (*E. coli*) produced an intermediate effect; the suppression of migration inhibition, although distinct, was not as pronounced as in the human LMI system. Two additional experiments gave similar results.

The antigen-induced MMI test is based on the following observations. Immunization of mice with syngeneic Moloney-lymphoma cells induces antibodies to viral proteins and virus-determined cell surface antigen (designated MCSA, that is, Moloney leukemia virus-determined cell surface antigen) (*22*). These mice develop transplantation immunity and generate killer cells against the target lymphoma. We have found that purified MCSA inhibits the migration of peritoneal cells from immunized mice (*23*).

We have tested the effect of the Namalwa IFN, mouse IFN, and IFN-α1 (*E. coli*) on the MCSA-induced MMI. As shown in Fig. 5, Namalwa IFN had no influence on migration inhibition, whereas mouse IFN abolished it almost completely, and the IFN from *E. coli* decreased it to an intermediate level. The effect of the IFN's on the MMI induced by antigen was stronger than on the MMI induced by mitogen. IFN-α1 (*E. coli*) thus shows a target cell range different from Namalwa IFN.

Inhibition of Daudi cell growth. We have compared the effect of authentic leukocyte IFN and IFN-α1 produced by *E. coli* on the multiplication of Daudi cells, and a line containing EBV derived from a Burkitt lymphoma, which is particularly sensitive to the action of human IFN (*24*). We used a new assay called Discotest, which was developed by two of us (J.G. and L.M.) and is described in (*25*). In short, Daudi cells are embedded in soft agarose, and a paper disk soaked with an IFN solution is placed on top of the agarose layer. The diameter of the halo of colony inhibition around the disk is proportional to the logarithm of IFN concentration, thus allowing an accurate determination of the cytostatic action of IFN.

The cytostatic effect of increasing concentrations of partially purified IFN-α1 (*E. coli*) and of authentic leukocyte IFN, mixed with an extract of nontransformed *E. coli* that had been subjected to the same purification procedure, was determined, and the cytostatic effect was plotted as a function of the antiviral titers. Figure 6 shows that the two dose-response curves are indistinguishable and that the ratio of antiviral to anticellular activity is practically the same for the two IFN preparations. Thus, IFN made in bacteria has the same ability to inhibit growth of Daudi cells as authentic IFN. The extract from nontransformed *E. coli* had no effect on Daudi cell growth.

The cell growth inhibition effect of IFN made in bacteria was neutralized by an antiserum to interferon (from sheep Gunnar), but only at a titer of antiserum to interferon three log units higher than authentic leukocyte IFN (data not shown). Perhaps the spatial configuration or the lack of proper glycosylation of bacterial IFN or both prevent its recognition by antibodies to native IFN. More likely, IFN-α1 was only a minor species or a species with lower antigenicity among the leukocyte IFN's against which the antiserum to interferon was raised.

The far more efficient neutralization of IFN from *E. coli* reported by Nagata *et al.* (*3*) was measured with the cytopathic-effect reduction assay, for which IFN from strains Hif-SN35 and Hif-280AH3 and a different preparation of antiserum (from sheep Ivar) to IFN was used.

Discussion

We reported earlier that *E. coli* transformed with any one of several plasmids containing human leukocyte cDNA produce a polypeptide capable of inducing resistance to virus in human cells.

Two separate cloned leukocyte IFN cDNA species designated IFN-α1 (*26*) and IFN-α2 (*27*), both capable of eliciting the formation of polypeptides with IFN activity in *E.coli*, have been isolated and sequenced; they differ in about 20 percent of their nucleotide residues, and it may be deduced that the cognate polypeptides differ in 17 percent of their amino acids. The deduced amino acid sequences of IFN-α1 and IFN-α2 differ in 25 and 20 percent, respectively, of their

Table 1. Increased reactivity of human blood lymphocytes after incubation with IFN-α1 (*E. coli*) or Namalwa IFN. The increase of cytotoxic activity against Daudi and Raji cells induced in lymphocytes from three normal donors was evaluated with a reactivity index, that is, the ratio of the number of untreated effector cells required to exert a certain degree of killing to the number of treated effector cells required to give the same degree of killing. Effector cells were pretreated with IFN-α1 (*E. coli*), Namalwa IFN, human leukocyte IFN (preparation PIF from K. Cantell) plus *E. coli* control extract, or *E. coli* control extract alone. All IFN preparations were added to a final concentration of 1000 U/ml; *E. coli* extract was added to a final protein concentration of 3 mg/ml. Spontaneous ^{51}Cr release from Daudi and Raji cells was 6 and 9 percent, respectively.

Donor	Target	*E. coli* control extract	IFN-α1 (*E. coli*)	Human Le IFN + *E. coli* control extract	Namalwa IFN
B. K.	Daudi	1	6.5	7.6	9.0
	Raji	1	3.0	5.5	9.0
N.K.	Daudi	1	4.8	4.3	5.7
	Raji	1	5.3	4.1	5.8
W.Y.	Daudi	1	4.2	4.0	6.1
	Raji	1	4.1	4.1	9.0

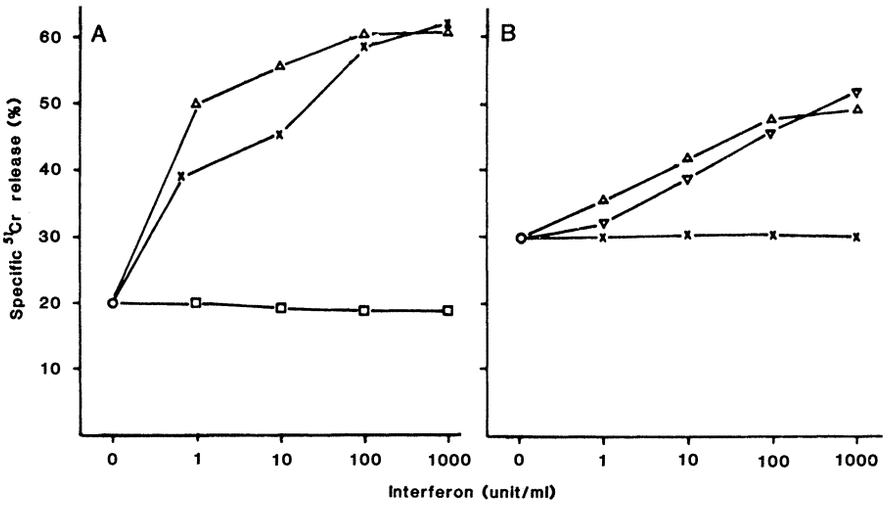
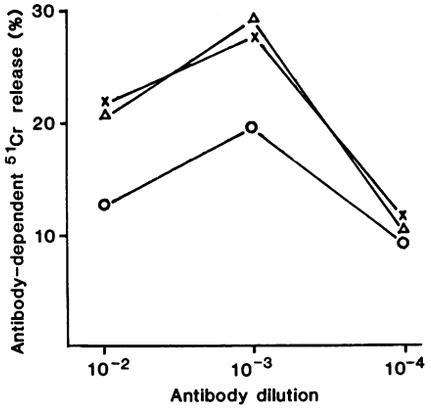


Fig. 2. Dose-response curve of the IFN effect on the cytotoxicity of human and mouse lymphocytes. Human and mouse effector lymphocytes, prepared as in Fig. 1, were first incubated for 2 hours in medium (○) (control); in medium containing different concentrations of IFN-α1 (*E. coli*) (△); *E. coli* control extract containing the same amount of protein as the IFN preparation from *E. coli* (□); human IFN prepared from Namalwa cells (×); or mouse IFN (▽). After incubation, the effector cells were washed and tested in a 4-hour cytotoxicity assay. (A) Cytotoxic activity of human lymphocytes against Daudi cells at an effector to target ratio of 25 to 1. (B) Cytotoxic activity of mouse lymphocytes against YAC-1 cells at an effector to target ratio of 50 to 1. Spontaneous release of ^{51}Cr was as described in Fig. 1.



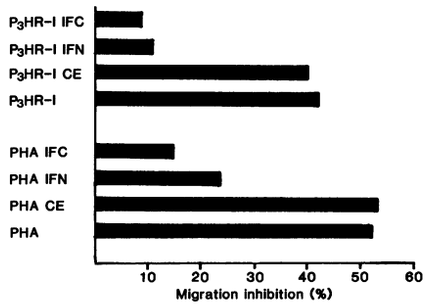
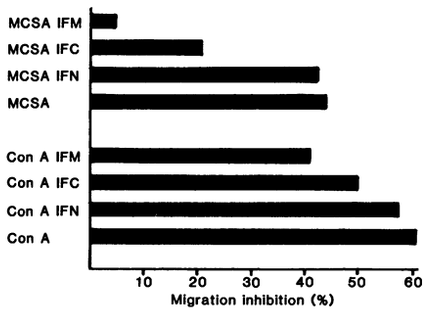


Fig. 3 (left). Effect of IFN treatment on antibody-dependent cell-mediated cytotoxicity. Human lymphocytes, prepared as described in Fig. 1, were incubated for 2 hours in medium (○); in medium containing 1000 international units per milliliter of HIFN-α1 (*E. coli*) (△); or Namalwa IFN (×). The cytotoxic activity (at an effector to target ratio of 33 to 1) was tested in a 4-hour ^{51}Cr release assay against GM, an EBV-transformed lymphoblastoid cell line derived less than 6 months earlier from the blood lymphocytes of a normal donor. The target cells were exposed to different concentrations of rabbit antiserum to human immunoglobulin (Dako) before addition of effector cells. Untreated target cells were insensitive to spontaneous and IFN-induced cytotoxicity. Spontaneous release of ^{51}Cr was 16 percent. Fig. 4 (right). Effect of IFN on human leukocyte migration inhibition induced by mitogen (PHA) and antigen (EBV). The direct agarose microdroplet technique (*36*) was used with minor modifications. Buffy coat cells (20×10^6) from a donor whose serum was positive to EBV were mixed with 135 μl of substrate, containing an equal volume of 0.4 percent agarose and double-strength RPMI 1640 medium, supplemented with fetal calf serum (20 percent). Droplets (2 μl) of the suspension were placed in migration chambers (Sterilin). After the droplets had solidified, the chambers were filled with either RPMI-1640 medium, supplemented with 10 percent fetal calf serum containing phytohemagglutinin (1 μg/ml) (purified PHA, Wellcome) or an extract from the EBV-carrying P$_3$HR-1 cells (protein concentration, 50 μg/ml), and incubated for 24 hours at 37°C in an atmosphere containing 5 percent CO_2. The migration areas were measured and the percentage of migration inhibition (MI) was calculated as 100× [1 − (mean migration area with antigen/mean migration area without antigen)](control). An MI of > 20 (antigen-induced LMI) or of > 50 (PHA-induced LMI) was considered significant. One hundred units per milliliter of IFN-α1 (*E. coli*) (IFC) or Namalwa IFN (IFN), or *E. coli* control extract (CE) (the same amount of protein as in IFN-α) were added where indicated.



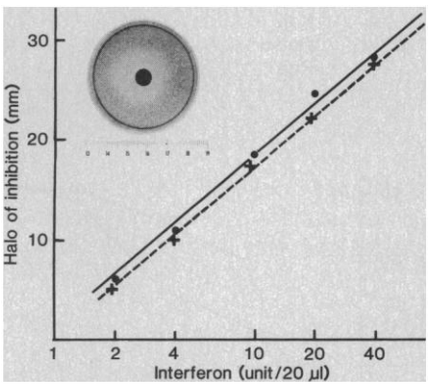


Fig. 5 (left). Effect of IFN on antigen- and mitogen-induced mouse macrophage migration inhibition (MMI). Mice (CBA-H2) were injected intraperitoneally with 5×10^6 irradiated (2000 roentgens) YBA-HR tumor cells induced by Moloney virus. After 24 days, 2.4 percent thioglycolate (1 ml) was injected into the peritoneal cavity. Four days later the peritoneal exudate cells, containing about 75 percent macrophages and 10 percent lymphocytes, were collected and used in the direct agarose microdroplet assay as described in Fig. 4. The migration chambers were filled with medium containing concanavalin A (Pharmacia) (10 μg/ml) or purified MCSA (Moloney cell surface antigen) (2 μg/ml) prepared as described (*37*). Where indicated, 100 international units per milliliter of mouse IFN (IFM), IFN-α1 (*E. coli*) (IFC), or Namalwa IFN (IFN) were added. Calculations were made as in Fig. 4. Fig. 6 (right). Dose-response curve of growth inhibition of Daudi cells in agarose by IFN-α1 (*E. coli*) and leukocyte IFN. Daudi cells were seeded in agarose medium (*25*). Paper disks were soaked with 20 μl of the IFN sample to be tested and placed on the cell overlay. Plates were incubated for 10 days in a controlled atmosphere, and the diameters of the inhibition halo were measured on photographs. The diameter of the halo, after subtraction of the diameter of the disk, was plotted as a function of the IFN concentration on the disk, expressed in international antiviral units per milliliter (solid line) IFN-α1 (*E. coli*); (dashed line) control preparation of authentic leukocyte IFN were mixed with bacterial control extracts (as described in Fig. 1) to give the same protein concentration as in the IFN-α1 (*E. coli*) preparation. The ratio of antiviral units to anticellular units was 3.3 to 1 for bacterial IFN and 3.9 to 1 for leukocyte IFN. One unit of cell growth inhibition activity is the concentration giving an inhibition halo of 10 mm. The inset is a photograph of a halo of inhibition given by 10 international antiviral units of bacterial IFN.

residues from that of the fragment of lymphoblastoid IFN sequence published by Hood and his colleagues (*28*). Therefore, we conclude that there are at least three distinct active IFN-α genes. In fact, analysis of human chromosomal DNA revealed the presence of at least eight distinct IFN-α- related genes (*29*).

We show in this article that a partially purified preparation of IFN-α1 produced by *E. coli* has the same effect as partially purified lymphoblastoid (Namalwa) IFN on human blood lymphocytes in regard to the enhancement of natural killing activity, antibody-dependent cell-mediated cytotoxicity, reduction of leukocyte migration inhibition, and inhibition of growth of Daudi cells. The activities of the two preparations in these tests, relative to their potency in the cytopathic-effect reduction assay, is indistinguishable. Since an extract of a control strain of *E. coli*, subjected to the same purification procedure as the extract containing IFN, showed no effect, the activity must be due to the IFN polypeptide encoded by the cloned IFN cDNA plasmid. These experiments add weight to the generally accepted view that the activities observed in the impure natural human IFN preparations are due to IFN itself and not to one or more associated contaminants. Moreover, it would seem unlikely that specific glycosylation of the natural IFN-α polypeptides plays a crucial role in the activities we have examined, since it is not to be expected that glycosylation of an IFN polypeptide in *E.coli*—if it occurs at all—would give rise to a modification similar to that imparted by human cells.

It is striking that IFN-α1 from *E. coli* is active on natural killer cells of the mouse, whereas lymphoblastoid IFN is not. Moreover, experiments carried out by Stewart and his colleagues (*30*) have shown that IFN-α1 (*E. coli*) is 20 to 50 times more active on bovine, cat, and mouse cells than on human cells in regard to its capacity to induce resistance to virus. In this regard, IFN-α1 (*E. coli*) resembles a component of natural leukocyte IFN detected by Linn and Stewart (*31*) after two-dimensional sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. A second species of α-type IFN that we have identified, IFN-α2, causes the formation in *E. coli* of a polypeptide whose activity as measured by the cytopathic-effect reduction assay, was less on bovine than on human cells (*27*), as is the case with unfractionated, natural leukocyte IFN. It will be of interest to see whether this target-cell specificity also extends to the other IFN activities.

It appears that leukocytes and lym-

phoblastoid cells may produce several different species of IFN that are encoded by distinct genes and may have distinctive biological properties; for example, in regard to their target-cell specificities. Cloning techniques should make each of these species available in quantity and allow their exact characterization. The results obtained so far are promising in regard to the possible replacement of natural IFN by products made in *E. coli*.

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Glossary

This list of ad hoc definitions is intended to be helpful to readers of the preceding articles. The definitions are not exhaustive.

acute transfection short-term infection of cells with DNA

ADCC antibody-dependent cell-mediated cytotoxicity

amber mutation a mutation in which a polypeptide chain is terminated prematurely; results from an alteration in a codon such that the codon becomes UAG, which signals chain termination

amplification 1. treatment (for example, chloramphenicol) designed to increase the proportion of plasmid DNA relative to that of bacterial DNA; 2. replication of a gene library in bulk

anticodon the triplet of nucleotides in a tRNA molecule that associates by complementary base pairing with the codon in the mRNA during translation

antiparallel describes molecules that are parallel but point in opposite directions (the strands of DNA are antiparallel)

antisense strand of DNA that has the same sequence as mRNA

AUG see initiation codon

blunt end DNA end with both tein (sometimes CRP or CGA); it paroverlapping sequence is exposed

cap the structure found at the 5′ end of many eukaryotic mRNA's; it consists of 7′-methyl-guanosine-pppX, where X is the first nucleotide encoded in the DNA; it is not present in prokaryotic mRNA's; it is added posttranscriptionally near the TATA (Hogness) box

CAP not to be confused with cap; CAP is catabolite gene activator protein (sometimes CRP or CGA); it participates in the initiation of transcription in prokaryotes

capsid the protein coat of a virion or virus particle

cg clone crown gall clone; cg clone 1, cg clone 2, and the like

C_H constant portion of the immunoglobulin heavy chain

chromosome walking sequential isolation of overlapping molecular clones so as to span large chromosomal intervals

cistron a DNA fragment or portion that specifies or codes for a particular polypeptide

class see immunoglobulin class

class switch a switch in the expression of a B lymphocyte from one antibody class to another

codon a group of three nucleotides that codes for an amino acid

cohesive termini (cohesive end) DNA molecules with single-stranded ends that show complementarity, making it possible, for example, to join end to end with introduced fragments

cold sensitive mutation mutation leading to a gene that is functional at high (permissive) temperature but inactive at low (restrictive) temperature

complementary DNA (cDNA) DNA that is complementary to messenger RNA; used for cloning or as a specific and sensitive probe in hybridization studies

consensus sequence an average sequence, each nucleotide of which is the most frequent at that position in a set of examples; used for RNA splice sites and other sites

cross hybridization hybridization of a probe to imperfectly matching (less than 100 percent complementarity) molecules

crossing-over exchange of genetic material between chromosomes that pair during meiosis (homologous chromosomes)

cut a double-strand scission in the duplex polynucleotide in distinction to the single-strand "nick"

episome a circular gene fragment

exon portion of DNA that codes for the final mRNA