CHEMISTRY AND BIOLOGY OF INTERFERONS: RELATIONSHIP TO THERAPEUTICS

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Recombinant DNA Production
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0224 REGULATION OF INTERFERON-ß AND IFN-ASSOCIATED GENES STUDIED AT THE MOLECULAR LEVEL, John Collins, Ulrich Mayr, Wolfgang Bruns, Hansjörg Hauser and Gerhard Gross, G.B.F., D-3300 Braunschweig, FRG.

We have recently reported that genes located on the human genome near the Interferon ß-gene are co-ordinately induced by poly I-C in human fibroblasts (1). These genes we refer to as "Interferon associated" (IA-genes). IA-genes are differentially regulated in Sendai virus induced lymphoblastoid cells. The response to induction is slow, being first detectable with cloned probes after about 90'. Further studies of the kinetics of induction revealed a small (about 5S) RNA species (isRNA) which responds immediately to induction in fibroblasts and disappears at later times. In mouse cell hybrids which can be induced to high levels of human Interferon ß production the human isRNA gene is also induced but isRNA accumulates even at later times. The presence of the human isRNA gene is not a prerequisite for human IFNß production in mouse cell hybrids.

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0225 EXPRESSION OF HUMAN IMMUNE (γ) INTERFERON IN HETEROLOGOUS HOST CELLS, David V. Goeddel, Rik Derynck, Ronald A. Hitzeman, David W. Leung, Arthur D. Levinson, Christian C. Simonsen, Elizabeth Yelverton, and Patrick W. Gray, Genentech, Inc., South San Francisco, CA 94080

Messenger RNA was isolated from human lymphocytes stimulated with staphylococcal enterotoxin B and desacetylthymosin α_1 and fractionated by gel electrophoresis. Fractions enriched for IFN-γ mRNA were used to prepare a cDNA colony library in E. coli. Clones containing "induced" cDNAs were identified by independent screening with two radiolabelled cDNA probes prepared using RNA isolated from mitogen induced or uninduced lymphocyte cultures. IFN-γ cDNA sequences were identified by expression of antiviral activity in three independent expression systems: in E. coli, utilizing the trp promoter, in yeast under control of the phosphoglycerate kinase gene promoter, and in monkey cells where expression was directed by the SV40 late promoter. The IFN-γ cDNA is 1200 bases in length and contains an open reading frame of 166 amino acids. This sequence encodes a putative signal sequence of 20 residues and a mature sequence of 146 residues (17,110 daltons). Like natural human IFN-γ the antiviral activity expressed in E. coli, yeast, and monkey cells is sensitive to treatment with acid (pH2) or detergent (0.1% SDS). Similarly, the cloned IFN-γ antiviral activity is neutralized by antiserum specific for natural IFN-γ, but not by antisera for leukocyte interferon (IFN-α) or fibroblast interferon (IFN-ß). Southern hybridizations demonstrate the presence of a single IFN-γ gene in human genomic DNA. This single IFN-γ gene appears to contain at least one intervening sequence.

0226 STURCTURE AND EXPRESSION OF THE CLONED HUMAN INTERFERON GENES, Tadatsugu Taniguchi, Shigeo Ohno, Chikako Takaoka and Tatsunari Nishi, Department of Biochemistry, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo 170, Japan.

Much information about human interferon (IFN) genes has been accumulated in the past few years. We have previously reported the cloning and the structural analysis of the human IFN-β_1 cDNA and a chromosomal segment containing the gene (1,2). Comparison of the cDNA sequence with that of the IFN-α_1, cloned and analysed by Weissmann and colleagues, indicated that the two genes were derived from a common ancestor (3). In addition, upstream of the putative transcription initiation site of the IFN-β_1 gene, we as well as others have found a unique sequence which shows homology to the IFN-α_1 counterpart.

In order to study further the mechanism of the IFN gene expression, we have introduced a cloned 1.8 kb chromosomal segment containing the IFN-β_1 gene into cultured mouse cells. The introduced IFN-β_1 gene was found to be expressed in the regulated manner. Work is in progress to identify unique DNA sequences which confer the inducer-responsiveness of the gene.

We have also constructed a series of recombinant plasmids which gave rise to the high level of IFN-β_1 synthesis in *E.coli* host by using several bacterial promoters.

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Cellular Mechanisms of Action and Genetics

0227 INTERFERON MODULATES CELL STRUCTURE AND FUNCTION, Igor Tamm, Eugenia Wang, Frank R. Landsberger and Lawrence M. Pfeffer, The Rockefeller University, New York, NY 10021

Interferon treatment alters the structure of the plasma membrane and the cytoskeleton in a variety of cells. These structural changes are associated with modulation of specific functions in human epidermoid carcinoma (HeLa-S3) cells, human skin fibroblasts (FS-4 and ME), and thioglycolate-elicited mouse peritoneal macrophages treated with homologous β interferon. The rigidity of the plasma membrane lipid bilayer is increased within 30 min from the beginning of treatment of HeLa cells growing in suspension, but returns to control level within a few hr(1). This transient increase may be related to triggering of the cascade of events that leads to the interferon-induced altered phenotype. Increased rigidity returns by 24 hr after beginning of treatment and persists. A marked increase in the abundance of submembranous actin filaments in interferon-treated HeLa cells is associated with impairment of the mobility of cell surface receptors for concanavalin A, slowing of cell proliferation, increase in cell size, and increased frequency of multinucleated cells (2-4; L.M.P. & I.T., unpublished observations). In interferon-treated fibroblasts, a marked increase in the organization of actin-containing microfilaments into bundles is associated with decreased cell locomotion, membrane ruffling, and intracellular movement of organelles, with prolongation of the intermitotic interval, increase in cell size, and increased frequency of multinucleated cells (4,5). No changes have been observed in the distribution of microtubules or 10 nm filaments in interferon-treated fibroblasts. In interferon-treated macrophages, a marked increase in the submembranous meshwork of actin filaments, notably in regions of phagocytic cups, is associated with increased efficiency of phagocytosis (6). However, microtubules and 10 nm filaments are not located near the phagocytic cups either in interferon-treated or control cells. In control cells they form an extended network, but in interferon-treated cells, microtubules and 10 nm filaments aggregate in the perinuclear region. In treated cells pinocytosis is suppressed, which appears to be linked to the disruption of the network of microtubules and 10 nm filaments.

We conclude that β interferon treatment leads to an increase in the organization of actin-containing microfilaments in widely different cell types. Although associated with depression in the motile and proliferative functions of aneuploid carcinoma cells as well as diploid fibroblasts, this increase appears to enable phagocytes to ingest particulate material more efficiently.

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0228 INTERFERONS AND GENE ACTIVATION: CLONING OF cDNA SEGMENTS COMPLEMENTARY TO MESSENGER RNA'S INDUCED BY INTERFERONS, P. Lengyel, H. Samanta, J.P. Dougherty, M.E. Brawner, and H. Schmidt, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

It was shown earlier that the treatment of cells with interferons results in the increased accumulation of certain mRNAs and of the corresponding proteins (1-5). We report here the isolation of a cDNA clone which is complementary to a mRNA whose level is increased in cultured cells after treatment with an interferon. Assays involving hybridization with this cDNA clone reveal that by 3 hours after beginning the exposure of mouse Ehrlich ascites tumor cells to 1000 units/ml of a pure mouse beta interferon (6,7) the level of an about 6×10^5 dalton mRNA is increased. Between 6 and 12 hours the increase is maximal (about 12 fold) and by 15 hours the level decreases, even if the cells are continuously exposed to interferon. The level of this mRNA is not enhanced by interferon in cells treated with actinomycin D.

The characterization of the gene specifying the mRNA in question and the protein into which this mRNA is translated are underway. We are also continuing the search for cDNA plasmids specifying further mRNAs induced by interferons.

The availability of such plasmids should make possible among others: (1) the chromosomal mapping of genes activated by interferons; (2) the comparison of the structures of such genes and (3) the correlation between the activation of particular interferon-responsive genes and particular manifestations of interferon action.

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0229 CONTROL PROCESSES IN INTERFERON PRODUCTION

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Three levels of control have been identified in interferon formation:

1. An overall on/off switch which determines whether interferon production takes place when cells are treated with an inducer.
2. A control process which activates the transcription of the interferon genes after treatment with an inducer.
3. Control of the amount of transcription of mRNA and its subsequent degradation.

The first level of control was discovered by the use of embryonal carcinoma cells[1]. The undifferentiated stem cells do not produce interferon after treatment with an inducer, but will do after differentiation of the cells. The process has now been studied in detail by use of a single-cell assay for interferon to show that the proportion of cells in a culture that can produce interferon rises as differentiation proceeds. A similar process occurs in early mouse embryos, where undifferentiated stem cells fail to produce interferon but the outer differentiated layers of the embryo do.

It has been widely assumed, although not proved, that the activation of interferon mRNA transcription is controlled by a nucleotide sequence upstream from the 5'-region of the interferon gene. Recently a substantial amount of sequencing data of such putative control areas has become available and will be reviewed.

Once transcription has been activated, it may be modulated in two ways - by increasing the rate of transcription ("super-production") and by increasing the half-life of the mRNA that is formed ("super-induction"). The former occurs when cells are treated with butyrate and 5-bromodeoxyuridine[2,3] while the latter occurs when cells are treated under certain conditions with metabolic inhibitors[4], or are incubated at temperatures below 37°C[5].

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0230 THE 2-5A (pppA2'p5'A2'p5'A) SYSTEM IN INTERFERON-TREATED AND CONTROL CELLS

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2-5A was discovered on the basis of its role in the interferon-mediated inhibition of cell-free protein synthesis by double-stranded RNA (dsRNA). This occurs by two mechanisms, (1) phosphorylation of the initiation factor eIF2 by a dsRNA and interferon mediated protein kinase, (2) activation of a nuclease by nanomolar concentrations of 2-5A synthesised by the 2-5A synthetase induced by interferon and activated by dsRNA. 2-5A introduced into intact cells inhibits protein and DNA synthesis and virus growth. Sensitive and convenient radioimmune and radiobinding assays capable of detecting nanomolar concentrations of 2-5A and related oligonucleotides have been developed and used to detect the natural occurrence of 2-5A in interferon-treated, EMC virus-infected mouse and human cells.

The 2-5A-dependent nuclease has an unusual specificity: it cleaves RNA on the 3' side of UN doublets to yield UpNp-terminated products. Cleavages are observed predominantly at UA and UU. The nuclease also cleaves rRNA in intact ribosomes to yield a characteristic pattern of products. As might be expected from the natural occurrence of 2-5A in interferon-treated EMC-infected cells rRNA from these cells showed the same distinctive products. Thus not only is 2-5A present but the 2-5A-dependent nuclease is active in interferon-treated, EMC-infected cells. In the absence of interferon treatment, however, the nuclease is lost or inactivated in response to infection indicating a further level of control in the 2-5A system. The inactivation of the 2-5A-dependent nuclease in response to infection could account for the fact that in the absence of interferon treatment the production of low levels of 2-5A does not prevent growth of EMC virus in L- or HeLa cells and the related paradox that EMC virus grows well in control HeLa cells despite their unusually high levels of 2-5A synthetase. It may be, therefore, that it is the prevention of the virus-mediated inhibition of the 2-5A-dependent nuclease rather than the induction of the 2-5A synthetase which is crucial for interferon to be effective in the activation of the 2-5A system in some virus-infected cells. No evidence was found for any similar EMC virus-mediated inactivation of the interferon and dsRNA-dependent protein kinase system.

The 2-5A system may have a wider significance. The synthetase and nuclease are widely distributed in a variety of cells and tissues and levels of the former at least vary with growth and hormone status suggesting that the system may also play some role in normal cell metabolism and the control of cell growth and development.

0231 SYNTHESIS AND ACTION OF INTERFERON RELATED TO THE CONTROL OF CELL FUNCTIONS.

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The multiple effects of interferons on cells appear to be mediated by induction of the synthesis of several classes of proteins among which are translation regulatory enzymes (e.g. 2'-5' oligoadenylate synthetase E) and surface antigens (e.g. HLA chains). The action of interferon on the synthesis of these proteins appear to be at the gene level since accumulation of mRNAs for the (2'-5') oligo A synthetase (1) and for HLA-A,B,C chains can be demonstrated using in vitro translation methods and hybridization to cloned cDNA probes. The HLA mRNA is increased by a factor of 5-10 fold and is already maximum from 2-4 hours after exposure of human lymphoblastoid cells or fibroblasts to IFN-α or β. The induction of HLA mRNA and the increase in surface HLA antigen seems to precede in time the increase in (2'-5') oligo A synthetase. IFN-γ induces HLA very efficiently as compared to its antiviral effect. The different human IFN species induce HLA and the (2'-5') oligo A synthetase to various extents in different cell types.

The function of the HLA increase due to IFN is still unclear (2), but it can be dissociated from the antiviral effect. The (2'-5')oligo A synthetase induction plays a role not only in IFNs antiviral effect but also in the inhibition of mitogenesis by IFN. Synthetic (2'-5')ApApA also inhibits mitogenesis and there are growth-dependent variations in both the 2'-phosphodiesterase and the (2'-5') oligo A synthetase. We recently found that the latter enzyme is increased when Friend mouse erythroleukemia cells enter the stationary phase and differentiate. This increase is due to a spontaneous production of low amounts of IFN by these cell cultures. The IFN produced during growth-transition is of type-I but differs in its biological and immunological properties from that induced by a virus in the same Friend cells cultures. It has a 50-fold higher antimitogenic effect.

We propose that some IFN species are synthesized to play a role in the multiple cell regulatory effects of IFN, while others are more directly responsible for the protection of cells against viral infections. This may explain the continued existence of the multiple IFN genes which appear to be active in human cells.

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Cell Biology of Interferons

0232 PRESENCE OF INTERFERONS IN HUMAN AMNIOTIC FLUID

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Since 1968 we have presented a number of reports showing that low concentrations of interferon are able to invert malignant cells to a normal phenotype. Such cells lose in parallel their capacity to grow in agar or produce tumors in animals. We have therefore explored the possible role of interferon during embryonic development in humans. Indeed, interferons of an α-type are continuously detected in the amniotic fluid from the first month of pregnancy to delivery (P. Lebon, S. Girard, F. Thépot & C. Chany, C.R. Acad. Sci. Paris, 293: 69-71, 1981; J. Gen. Virol., in press). The origin of this interferon is unknown but could stem either from fetal cells or from embryonic annexes.

We have presently studied different interferons induced by Sendaï virus in the amniotic membrane. After improvements by means of theophylline and priming, we obtain regularly 10,000 - 20,000 international units (IU)/ml and between 10^6 - 10^7 IU/membrane.

The analysis of different interferons produced by this method shows a considerable heterogeneity and reveals to our great surprise a number of interferons not yet described, belonging either to the α, β, or intermediate types. After SDS gel electrophoresis, their molecular weight ranges from 19K - 80K. The possible biological significance of these findings will be discussed.

0233 EFFECT OF INTERFERON ON CELLULAR ENZYMES, T. Sreevalsan, E. Lee, T. Butt* and R. Cihlar, Department of Microbiology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007 and NIADDKD, National Institute of Health, Bethesda, Md. 20014

Quiescent Swiss mouse 3T3 cells can be stimulated to enter S by growth factors or serum (1) When added at the time of stimulation, mouse interferon (IFN) or sodium butyrate inhibited the initiation of DNA synthesis. Since butyrate has been reported to enhance IFN action in transformed cells (2) we determined whether such was the case in normal 3T3 cells. When used in combination, butyrate, acting in a synergistic fashion, enhanced the inhibitory effects of IFN on cellular DNA synthesis.

Experiments designed to assess the bases of the observed synergism between IFN and butyrate produced the following novel findings. 1) Butyrate was effective in inhibiting DNA synthesis in cells passing from $G_0 \rightarrow S$ as well as in cells exiting through G_1 period directly following mitosis. However, IFN could inhibit DNA synthesis only when cells were transiting from $G_0 \rightarrow S$. 2) Protein methylation, an event occurring in cell passage from $G_0 \rightarrow S$ was inhibited by IFN but not by butyrate. 3) The induction of ornithine decarboxylase, apparently another event characteristic of cells passing from $G_0 \rightarrow S$ was inhibited by IFN but not by butyrate. 4) The induction of poly ADPR synthetase, a late G_1 event was inhibited by IFN or butyrate.

Based on these results we conclude that IFN inhibits DNA synthesis in quiescent 3T3 cells by inhibiting critical events necessary for cell passage from $G_0 \rightarrow G_1$ while butyrate inhibits events needed for $G_1 \rightarrow S$ transit.

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0234 DOUBLE STRANDED RNA AND 2',5'-OLIGOADENYLATE: COMPANIONS IN INTERFERON ACTION? Paul F. Torrence[1], Jiro Imai[1], Krystyna Lesiak[1], Margaret I. Johnston[1], Helmut Jacobsen[2], Robert M. Friedman[3] and Brian Safer[4], Laboratories of Chemistry[1] and Experimental Pathology[2], NIADDK, Laboratory of Molecular Hematology[4], NHLBI, NIH Bethesda, Md. 20205 and Department of Pathology[3], Uniformed Services University of the Health Sciences, Bethesda, Md.

Analogs of dsRNA and of 2-5A and its derivatives can provide the means to dissect the relative importance of the protein kinase pathway versus the 2-5A pathway as mediators of the dsRNA-induced inhibition of translation in extracts of interferon treated cells.

2-5A trimer core monophosphate, p5'A2'p5'A2'p5'A, has been shown to prevent the inhibition of translation and degradation of RNA caused by 2-5A. Structure-activity relationships for this antagonistic action have shown that the 5'monophosphate moiety is required for antagonistic activity. In contrast, the 2' terminal ribose is not required since it can be extensively modified without decrease in antagonistic capacity; however, complete removal of the 2' terminal adenosine leads to a significant, albeit not total, loss of antagonistic capacity, implying a function of the 2' terminal adenine moiety in endonuclease binding. The 2-5A-activated endoribonuclease will also tolerate the substitution of a 3',5-phosphodiester bond for at least one of the 2',5'-phosphodiester bonds without complete loss of antagonistic capacity. Chemical modification can potentiate the ability of oligonucleotides to reverse the action of 2-5A, and this increase in potency correlates with an increase in stability to degradation by enzyme activities present in cell-free extracts. Furthermore, the relative behavior of oligomers as 2-5A antagonists provides a guide to structures that will have increased protein synthesis inhibitory potency compared to 2-5A itself.

Up to 80-90% of the inhibition caused by poly(I)·poly(C) in extracts of interferon-treated L cells can be relieved with 2-5A derivatives such as 2-5A trimer core monophosphate, suggesting that the major mechanism mediating the translational inhibitory properties of poly(I)·poly(C) in extracts of interferon-treated cells is the 2-5A system.

The dsRNA analogue, poly(A)·poly(dUfl)[poly(2'-fluoro-2'-deoxyuridylic acid], is not an activator of 2-5A synthetase but does activate the protein kinase of both rabbit reticulocyte lysates and interferon-treated mouse L cell extracts to phosphorylate eIF-2α as well as the protein P_1. Analysis of 32P-phosphopeptides produced by proteolytic digests show that phosphorylation induced by poly(I)·poly(C) and poly(A)·poly(dUfl) in mouse cell extracts occurs on the same peptide(s) of eIF-2α. However, in contrast to the situation in rabbit reticulocyte lysates wherein both poly(I)·poly(C) and poly(A)·poly(dUfl) are potent inhibitors of protein synthesis, in extracts of interferon-treated L cells, only poly(I)·poly(C), but not poly(A)·poly(dUfl), causes inhibition of protein synthesis. These data show that eIF-2α and/or protein P1 phosphorylation does not correlate with inhibition of protein synthesis by poly(I)·poly(C) in extracts of interferon treated L cells.

Relationship to Immunity

0235 PROPERTIES OF HUMAN γ INTERFERON

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We have devised a method for producing large amounts of human γ interferon in lymphocyte cultures stimulated by PHA. Titers were usually in the range of 10.000 to 30.000 u/ml.

Crude γ interferon can be adsorbed on silicic acid, from which the antiviral activity is eluted by a buffer containing ethylene glycol and a high salt concentration. This treatment allows quantitative recovery of γ interferon with a specific activity of 5-10 x 10^6 u/mg of proteins.

Chromatography on several sorbents (Blue-Sepharose, decyl-agarose, poly I-agarose, Con A-Sepharose and Sephacryl S 200) as well as chromatofocalization analysis enabled us to ascertain some of the physicochemical properties of γ interferon, including molecular heterogeneity, isoelectric points, M.W. and apparent hydrophobicity.

We also tested γ interferon preparations for lymphokine activities during different fractionation steps. Although such activities were present in interferon eluted from silicic acid, it was possible to dissociate them from the antiviral activity using certain chromatographic sorbents.

The biological activities of semi-purified γ interferon will be discussed.

0236 INDUCTION OF IMMUNE INTERFERON FROM HUMAN LYMPHOCYTES BY INFLUENZA INFECTED STIMULATOR CELLS.

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We recently reported that influenza infection of humans induced interferon and augmented levels of natural killer cell activity.[1] We also detected a virus specific HLA restricted cytotoxic T lymphocyte response in humans given live attenuated or inactivated influenza vaccines.[2] In addition to the virus specific HLA restricted lysis by the volunteers' lymphocytes of ^{51}Cr labelled target cells, we noted a lower degree of non-HLA restricted lysis which was not virus specific.[2] Since it had been reported that influenza infection *in vivo*[3] and *in vitro*[4] could induce interferon, we speculated that the nonspecific cytotoxicity was due to augmented natural killer cell activity by the cultured lymphocytes as a result of interferon production in the culture. The titers of interferon produced by the lymphocytes stimulated by influenza infected stimulator cells were >10,000 U/ml. The interferon was pH 2 labile and was not neutralized by antisera to alpha or beta interferon.[5] It did not bind to a monoclonal antibody to alpha interferon, and was neutralized by antiserum to gamma interferon. The influenza antigen responsible for stimulating this response is a crossreactive determinant present on all human and non-human influenza viruses tested, but preparations of purified hemagglutinins do not induce this immune interferon.

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Role in Human and Animal Disease

0237 INTERFERON IN SYSTEMIC LUPUS ERYTHEMATOSUS. Olivia T. Preble[1], John H. Klippel[2], David Koffler[3], Jan Vilcek[4], and Robert M. Friedman[1], Dept.of Pathology[1], Uniformed Services University of the Health Sciences, Bethesda, Md., Arthritis Branch[2], NIADDK, NIH, Bethesda, Md., Hahneman Medical School[3], Philadelphia, Pa., and Dept. of Microbiology[4], New York University Medical Center, New York, N.Y.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by a disfiguring skin rash, arthritis and nephritis. Individuals with SLE may have circulating immune complexes, or antibody to ss or ds DNA or to several small cellular ribonucleoprotein complexes. Many patients with SLE also have high levels of circulating interferon (IF). Other workers reported that the IF in SLE patients was "immune", or gamma, IF based on its instability at pH=2. We have examined the relationship between IF and SLE, using more specific criteria to characterize the IF. We found that an unusual acid-labile subspecies of human leukocyte or alpha IF is the predominant IF in SLE patients.

Circulating IF was undetectable ($\leq$4 Int. units/ml) in serum from 22 healthy controls or from 13 patients with drug-induced SLE. In contrast, 61 out of 138 patients with autoimmune SLE had significant titers of IF (8-128 Int. units/ml) in their serum. Although there was a rough correlation between overall disease activity and IF (52.3% of the patients with active disease had serum IF, compared with only 25% of those with disease in remission), no good correlation was found between individual serologic or clinical markers of disease activity and the presence or absence of IF. Serum from IF-negative patients did not neutralize reference human alpha, beta, or gamma IF, and pretreatment of human fibroblasts with IF-negative SLE sera did not inhibit development of the antiviral state.

The biological properties of the serum IF in SLE patients were compared to the properties of reference human alpha, beta and gamma IF. All of 79 SLE-IF samples tested had similar antiviral activities on bovine kidney and human (GM2504) fibroblast cells. This is characteristic of human alpha, but not beta or gamma, IF. In addition, 30 out of 33 SLE-IF samples were neutralized ($\geq$4 fold) by highly specific rabbit anti-IF alpha antiserum and not by antiserum to beta IF. Furthermore, plasma IF from one SLE patient was also completely bound to an anti-IF alpha affinity column. However, the alpha IF in 23 out of 30 samples (76.7%) was highly sensitive ($\geq$4-fold inactivation) to incubation at pH=2. The IF in 8 of the pH sensitive samples was also inactivated at 56°. Multiple IF samples from the same patient had similar properties but IFs from different SLE patients were not all identical.

Unstimulated mononuclear cells purified from IF-positive SLE patients did not spontaneously release IF in vitro. Pokeweed mitogen, but not PHA, induced high titers of gamma IF in cells from both IF-positive and IF-negative SLE patients. UV-inactivated NDV induced large amounts of alpha interferon, whereas 20 g/ml of poly I-C was ineffective in inducing IF under the same conditions.

0238 INTERFERON IN HUMAN AUTOIMMUNE DISEASES AND IN LYMPHOPROLIFERATIVE DISORDERS John J. Hooks, NIH, Bethesda, Md. 20205

Interferon (IFN) is recognized as a product of lymphoid cells which regulates immune responses. Since the study of altered immune states frequently provides insights into mechanisms of immunologic control, we looked for the presence of IFN in several human diseases associated with dysfunctions in immunoregulation. IFNs were present in two types of disorders, 1) autoimmune connective tissue diseases, and 2) a lymphoproliferative disease (T cell chronic lymphocytic leukemia - CLL). IFN is present in the sera of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, scleroderma, Sjogren's syndrome and vasculitis.[1] Moreover, the presence of IFN in SLE patients correlates with immunologic and clinical disease activity. This IFN is a leukocyte product (neutralized by anti-IFN-alpha) consisting of subtypes of IFN-alpha or a mixture of IFNs. The continued presence of IFN may be one of the factors contributing to the immunoregulatory dysfunctions observed in SLE.

IFN may also be involved in a second type of human lymphoid cell disorder. Recently, we reported a CLL patient with a disease characterized by proliferation of T cells with Fc receptors for IgG (T_G). However, unlike cells from normal individuals or from patients with other lymphoid malignancies, the patient's cells spontaneously produce IFN-gamma in vitro.[2] The peripheral lymphocytes consisted of 95% T_G cells which were OKT3+, OKT8+ and OKT4- and 3A1-. Functionally, the cells have normal or even elevated levels of antibody-dependent cellular cytotoxicity (ADCC) activity, low levels of natural killer (NK) activity and no suppressor activity. It is conceivable that IFN-gamma, which has been shown to trigger proliferation and/or differentiation of T_G cells, is the underlying stimulus for the proliferation of T cells in this patient.

Since IFN has multiple regulatory actions on lymphoid cells and immune responses, these studies point to the possibility that IFN might play a regulatory role in certain immunologic disorders and/or lymphoid malignancies.

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0239 INTERFERON INDUCED ENZYME ACTIVITIES IN VIVO. Bryan R.G. Williams and Stanley E. Read. Division of Infectious Diseases, Hospital for Sick Children, Toronto, Ontario, Canada.

The interferon-induced enzyme ppp(2'p5'A) synthetase (2-5A synthetase) can be detected in extracts of human mononuclear cells from healthy young adults. The treatment of these cells in vitro with interferon results in a several-fold increase in enzyme activity. Assays on mononuclear cells separated into T-cell and B-cell rich populations show the B-cells to have up to 40 times the 2-5A synthetase levels found in T-cells but the levels in both populations can be elevated by interferon treatment. Cells prepared from leukemic patients (acute or chronic lymphatic) show a low to normal range of enzyme activity which can be enhanced by interferon treatment. In contrast, the enzyme levels in cells from children with severe combined immunodeficiency disease (SCID) and defined blocks in T-cell function could not be enhanced by interferon treatment. Furthermore, unlike normal mononuclear cells the natural killer cell subpopulations from SCID patients were refractory to interferon stimulation. This refractory state appears to be limited to hemopoietic cells since skin fibroblast cell lines established from these children show an enhanced 2-5A synthetase response to interferon treatment. Whether this lack of response to interferon reflects the absence of an interferon-reactive lymphocyte subpopulation or the lack of interferon receptors on the lymphocytes is currently under investigation. However, these results may be indicative of a role for the interferon system in the differentiation of immunoregulatory T lymphocyte subpopulations. Interferon may also play an immunoregulatory role in fetal allograft survival since elevated levels of 2-5A synthetase can be detected in 80% of women tested from 12 weeks gestation to parturition.

Elevated levels of 2-5A synthetase are present in cells from patients with viral infections but not in those with bacterial infections. The enzyme assay is particularly useful in distinguishing bacterial from aseptic meningitis. Patients suffering viral infections while on immunosuppressive therapy do develop an enzyme rise but this is often delayed. Vaccination with live attenuated or killed virus vaccines results in elevated 2-5A synthetase levels over a prolonged period. High enzyme levels can also be detected in mononuclear cell extracts from patients during the acute erythroblastopenic crisis stage of aplastic anemia. This correlates with the presence of serum interferon and is further evidence for the involvement of viral infection and interferon activity in the etiology of this disease.

0240 IMMUNODEFICIENCIES, ANEUPLOIDY, AND INTERFERON, Lois B. Epstein, Jon Weil and Charles J. Epstein, Cancer Research Institute and Department of Pediatrics, University of California, San Francisco, CA 94143

Disease states in which interferon production has been shown to be depressed or enhanced will be discussed (1). In addition, we shall discuss Down syndrome (trisomy 21) in which abnormalities of the response to interferon have been demonstrated with regard to the peptides induced by interferon (2), and to the antiviral (3), antiproliferative (4) and antimaturational effects of interferon (5). An analysis of whether these abnormalities of production or response to interferon have significance in terms of the pathophysiology of disease processes will be made.

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Pharmokinetics, Animal Models, and Clinically Predictive Studies Part I

Sponsored by Cetus/Shell Interferons Program

0241 INHIBITION OF TRANSPLANTED HUMAN OSTEOSARCOMA (HOS) TUMORS IN ATHYMIC MICE BY HUMAN INTERFERON (HuIFN). Lowell A. Glasgow, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah 84132.

HuIFN is being evaluated as a potential therapeutic agent for human malignancies. Unfortunately clinical trials are being initiated with minimal background data from preliminary studies in animal models. The major reason for this is the relative species specificity of HuIFN resulting in its lack of efficacy in non-primate experimental models. We developed an experimental model utilizing the sc transplantation of HOS cells into athymic mice. Prior to initiation of experiments in vivo the sensitivity of several lines of human tumor cells was determined in vitro. HOS cells were shown to be inhibited in culture by lymphoblastoid HuIFN (Lybl) and recombinant DNA HuIFN (-2). Utilizing a growth inhibition assay quantified by ^{51}Cr labeling, HuIFN (Lybl) inhibited HOS cells (512u-94%, 128u-87%, 32u-63%, 8u-36%, 2u-18%). In contrast a human melanoma cell line was resistant to the antiproliferative effect of HuIFN (512u-10%, 128u-5%, 32u-0, 8u-0). Athymic mice were inoculated with 4×10^6 HOS cells sc in the back, HuIFN (Lybl) was administered sc at the site of tumor cell inoculations once daily for 7d beginning 1d after the tumor cells. HuIFN completely prevented or inhibited the growth of HOS tumors in 4 experiments (control-35 tumors/35 mice, 50,00u-2/30, 25,000u-1/12, 12,500u-4/12, 6,250u-4/6). Following the same protocol the in vitro resistance of the melanoma cells was also reflected in vivo (control-6 tumors/6 mice, 100,000u-5/6, 50,000u-6/6, 25,000u-6/6). We postulated that because of species specificity the activity of HuIFN (Lybl) against human tumor cells in the in vivo model would reflect only the expression of antiproliferative activity and that NK cells would not be augmented and macrophages would not be activated in the mouse. This hypothesis was confirmed by the demonstration that in vitro HuIFN (Lybl) augmented human NK cell (21% specific ^{51}Cr release in controls was increased to 75% in the HuIFN group), but failed to augment murine NK cells. Furthermore, human monocytes were activated by HuIFN (Lybl) to be cytotoxic for HOS cells, but (HuIFN (Lybl) failed to activate tumor cell cytotoxicity by murine macrophages. A similar series of experiments was carried out with HuIFN -2 In summary the in vitro sensitivity or resistance of two human tumor cell lines to the antiproliferative activity of HuIFN was reflected in vivo in the athymic mouse model. The inability of HuIFN to augment murine NK cells or to activate murine macrophages in vitro suggested that the antitumor activity of HuIFN in this model was due to a direct antiproliferative effect on the human tumor cells.

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0242 THE EFFECTS OF NATURAL AND BACTERIAL DERIVED INTERFERONS IN RHESUS MONKEYS, Huub Schellekens, Primate Center TNO, Rijswijk, The Netherlands.

The high cost and scarcity of interferon has limited not only trials in man but also studies in experimental animals. There is only a very small number of studies being conducted on the antitumor and antiviral effects of interferon in animal models. Much more data from animal studies are essential for evaluation of the therapeutic potential of interferon in man. To broaden our knowledge on the application of interferon, we have been doing studies in rhesus monkeys over the years. The properties of human interferon can be directly evaluated in these animals. We study its effect on intradermal infection of these monkeys with vaccinia virus. We have experience now with about 300 animals. We have established that: 1) the optimal dose of HuIFN alpha is 500,000 $IU.kg^{-1}$ i.m.; 2) HuIFN alpha is more active than HuIFN beta; 3) HuIFN beta is not more active i.v. than i.m.; 4) IFN has to be injected before infection; 5) one injection every 5 days seems sufficient; 6) aspirin and immunosuppressive treatment have no effect on the efficacy of bacterial derived HuIFN alpha 2. This was as active as naturally derived HuIFN alpha 2 but did induce fever or leukopenia as does naturally derived HuIFN alpha 2. HuIFN alpha 2 was devoid of any toxicity even at a dose of 260×10^6 $IU.kg^{-1}$ intravenously.

0243 INTERFERONS AND DRUG METABOLISM, Gerald Sonnenfeld and Donald E. Nerland, Department of Microbiology and Immunology and Department of Pharmacology and Toxicology, Univ. of Louisville School of Medicine, Louisville, KY 40292

Interferon is now being rapidly developed as an antiviral and antitumor agent. Although originally described as solely an antiviral agent, interferon has now been shown to have several additional activities, including regulation of immune responses, regulation of cell growth and division, and regulation of cell surface antigen expression. Several ongoing experimental and clinical trials suggest great potential for interferon as a therapeutic agent for various chronic viral infections and for tumors. These therapeutic effects of interferon are perhaps due to the non-directly antiviral activities of interferon. Preliminary data from clinical trials and from several experimental studies suggest that administration of alpha/beta interferon inducers can inhibit the liver microsomal cytochrome P-450 drug metabolism system and the metabolism of drugs by that system. Studies with administration of highly purified alpha/beta interferon produced by E. coli containing genes for human alpha/beta interferon have shown similar results when the alpha/beta interferon was given in high concentrations (Singh, Renton and Stebbing, Personal Communication). This depression of drug metabolism could lead to prolonged retention of a drug by individuals receiving both interferon and drug therapy, perhaps resulting in synergistic effects of the drug and interferon or perhaps resulting in severe complications of the drug therapy. We have been able to show that induction of gamma interferon in mice also resulted in the specific suppression of the cytochrome P-450 system and the inhibition of the metabolism of the drug diphenylhydantoin. The degree of depression correlated with the level of gamma interferon induced. In addition, passive transfer of small amounts of exogenous gamma interferon to normal mice also resulted in depression of cytochrome P-450 and of the metabolism of diphenylhydantoin. The effects of interferon on drug metabolism may be modulated by some immunological effect, and the more potent effects of gamma interferon on drug metabolism may be due in part to a more potent immunoregulatory activity of the gamma interferon preparation. Preliminary data suggest that gamma interferon-treated mice had a trasient decrease in circulating iron levels three hours after gamma interferon induction. This transient decrease appears significant for several reasons. First, the decrease in serum iron coincides with the peak of gamma interferon induction. Second, administration of exogenous iron has been shown to cause a decrease in the level of hepatic cytochrome P-450 and an increase in microsomal heme oxygenase, the rate-limiting step in heme degredation. Third, administration of interferon inducers causes a decrease in cytochrome P-450 and aminolevulinate synthetase, the rate controlling step of heme synthesis, and an increase in heme oxygenase. It therefore is reasonable to hypothesize that gamma interferon induction causes a transient decrease in serum iron levels. The translocation of iron to the liver then may result in a decrease in the level of cytochrome P-450.

0244 STRUCTURE-FUNCTION STUDIES ON HUMAN LEUKOCYTE INTERFERON, Ronald Wetzel, Howard L. Levine, David A. Estell, Steven Shire, Protein Biochemistry Department, Genentech, Inc., So. San Francisco, CA 94080, and Thomas Bewley, Hormone Research Lab, University of California, San Francisco, CA 94143

Expression of high levels of human leukocyte interferons in E. coli has made available enough purified protein to undertake structure-function studies. Subtype A (LeIF-A) has the amino acid analysis, sequence and MW expected from the cDNA sequence. The molecule's disulfide bonds (Cys-1 to Cys-98 and Cys-29 to Cys-138) were elucidated by HPLC/tryptic mapping. CD and UV spectroscopy show the molecule to have features typical for a globular protein, including a densely-packed hydrophobic interior. The molecule contains 70% α-helix and less than 15% β-sheet by CD, consistent with secondary structure predictions. On titration to pH 2, LeIF-A undergoes a major reversible conformational change, including loss of most of its α-helix. At the same time its sedimentation coefficient changes from a value expected of a dimer or trimer at neutral pH to that of a monomer at pH 2. Reduction of the disulfide bonds of LeIF-A greatly reduces its solubility and irreversibly destroys its antiviral activity.

A series of interferon derivatives were tested for antiviral activity in an in vitro CPE assay, for antigenic activity in an ELISA assay based on two monoclonal anti-interferon antibodies, and for receptor binding activity in competition with high specific activity in vivo radiolabeled E. coli LeIF-A. Isolated fragments from trypsin digestion of LeIF-A, including a 54 amino acid peptide containing the Cys-1 to Cys-98 disulfide, had no activity in any of these assays. When both interferon disulfides are broken in such a way that the product remains soluble, antiviral activity is still destroyed. Only the Cys-29 to Cys-138 bond seems necessary for activity, however, since interferon subtype D lacking Cys-1 remains active. This Cys-29 to Cys-138 bond is likely to be the sole disulfide of fibroblast interferon, which competes effectively for the LeIF-A receptor in the binding assay. Modification of the two tryptophans of LeIF-A by several specific reagents dramatically reduces its antiviral, antigenic and receptor-binding activity, but does not greatly alter its α-helix content as measured by CD. Several discrete limited proteolysis products, including a derivative lacking the C-terminal 12 amino acids, had undiminished activities in all three assays.

These observations will be discussed in terms of a working model for interferon structure-function relationships.

Pharmokinetics, Animal Models, and Clinically Predictive Studies Part II

0245 PHARMACOLOGY OF RECOMBINANT-DNA DERIVED HUMAN INTERFERONS, N. Stebbing, S.H. Lee, J. Moore, and P.K. Weck, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080

The existence of a family of distinct but structurally related leukocyte interferon sub-types (1) has allowed investigation of various pharmacological properties of potential clinical use in the treatment of viral and neoplastic diseases. Many of the properties attributed to impure natural interferon preparations have been demonstrated with highly purified, recombinant-DNA derived materials. Effects of interferons on immune parameters, particularly antibody synthesis, appears to play an important role in antitumor effects and such effects also appear to be involved in interactions of interferons with chemotherapeutic agents. Such studies with human interferons have been possible in mice because one human leukocyte interferon sub-type (LeIF-D) is active in mice and a hybrid interferon (LeIF-AD) appears to be as active in mouse sytems as natural mouse interferon (2,3). Interferons can suppress hepatic P-450 metabolism in mice and effects on the efficacy of various drugs will be discussed. These studies imply that only certain aspects of the hepatic oxygenase system are affected by various interferons. Some of the demonstrated effects of interferons occur over distinct dose ranges and the various cloned interferons differ in several of their pharmacological properties.

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0246 INTERFERON PROPHYLAXIS FOR SIMIAN VARICELLA INFECTION IN ERYTHROCEBUS PATAS MONKEYS, Ann M. Arvin, David P. Martin, and Thomas C. Merigan, Stanford University School of Medicine, Stanford, CA 94305 and Litton Bionetics, Inc., Kensington, MD 20795.

Several epizootics of varicella-like infection have occurred in non-human primates including patas and African green monkeys and macaque species.(1) Using serologic techniques, the virus strains isolated from these outbreaks are closely related antigenically.(2) These viruses are also antigenically similar to human varicella-zoster virus in vitro. In vivo, immunization with human varicella-zoster virus provided protection against inoculation with a simian varicella (Delta herpes virus) strain.(3) Simian varicella is associated with a vesicular exanthem, visceral dissemination and a high mortality rate. Because of these clinical manifestations and the antigenic similarity of the viruses, simian varicella provides an animal model for disseminated varicella in immunocompromised patients. The occurrence of an epizootic in a patas monkey colony allowed us to evaluate human leukocyte interferon (HuIFN-a) prophylaxis in this model using the same regimen that is being tested for varicella prophylaxis in children with malignancy.

In this study, HuIFN-a prophylaxis was shown to be effective in a double-blind, placebo controlled trial during a naturally occurring outbreak of simian varicella. HuIFN-a was given at a dose of 5×10^5 units/kg/day beginning eight days after the first case had occurred. Control animals received normal saline + 5% albumin. The treatment course was five days. 14 male and 14 female animals were studied. Male and female animals were randomized separately because the initial case was in the male animal care area. During a fourteen day observation period that started with the first day of treatment, the attack rate in HuIFN-a treated animals was 2/14 compared to 9/13 in placebo recipients. ($p < .025$, X^2) One female placebo animal died of unrelated causes. One of 7 HuIFN-a treated males became ill compared to 7 of 7 control males ($p < .01$, X^2). Seven of 9 infected control animals died while both HuIFN-a treated animals with symptoms survived. Serum interferon levels ranged from 175-788 units in HuIFN-a recipients and were less than 10 units in placebo animals. Antibody titers to the virus isolated during the outbreak were measured by radioimmunoassay and were similar in HuIFN-a and placebo recipients. These results support continued study of HuIFN-a prophylaxis for human varicella-zoster virus.

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Clinical Trial Results I — Antiviral and Antitumor

0247 EFFECT OF INDUCER SCHEDULING ON THE LEVELS AND TYPES OF INTERFERONS STIMULATED IN VIVO, Dale A. Stringfellow, Sheldon D. Weed, Harold C. Vanderberg, and Roger Brideau, Experimental Biology Research, The Upjohn Company, Kalamazoo, MI 49001

The phenomena of hyporeactivity that develops in animals following multiple doses of an interferon inducer is well-characterized. Although the general observation is that animals progressively lose their ability to respond to repeated doses of the same inducer, these same animals are in many cases still capable of responding to unrelated inducers. With some compounds such as 2-amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP), which does not stimulate high circulating levels of interferon, an actual enhancement or priming effect on further induction by itself or other inducers was observed. In addition, the type of interferon induced varied with inducer, scheduling, and tissues evaluated. After a single injection of AIPP, serum interferon (α) levels of 50 to 100 units/ml were observed; a second dose one day later induced 400 to 500 units of α interferon/ml. The interferon levels detected in tissues such as the spleen varied with inducer and in many cases did not correlate with serum interferon levels in the same animals. For example, after daily doses of AIPP the interferon detected in spleen tissue was predominantly pH labile γ interferon while serum contained mostly pH-stable α interferon. These data illustrate that a) the levels of interferon induced depend upon the tissues evaluated, inducer, and dosage schedule used, and b) serum interferon levels may not reflect the actual conditions at the tissue or organ level.

0248 INTERFERON IN RENAL TRANSPLANT RECIPIENTS, Martin S. Hirsch, Robert T. Schooley, Robert H. Rubin, Department of Medicine, Massachusetts General Hospital, Boston, MA 02114

Cytomegalovirus (CMV) infection is an important limiting factor in the success of renal transplantation. CMV can cause disease syndromes by itself (fever, leukopenia, glomerulonephritis) or can predispose to severe complications such as fungal or bacterial superinfection and possibly cancer. Although primary CMV infections in transplant recipients are more often associated with complications than are reactivation infections, the impact of both types is great.

We are conducting double-blind, placebo-controlled trials of interferon prophylaxis in renal transplant recipients subject to either primary or reactivation CMV infection. Earlier studies of ours indicated that a 6 week course (36 million units) of interferon-α could reduce CMV viremia, delay CMV viruria, and both delay and diminish Epstein-Barr virus excretion (1,2). Current studies involve a 14 week course (102 million units) of interferon-α prepared by Dr. Kari Cantell. Recipients are monitored for viral infections by clinical and laboratory studies, for complications of infection, for immune function (NK cell cytotoxicity, T lymphocyte subsets), and for drug toxicity.

An updated summary of results from these trials will be presented.

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0249 CLINICAL STUDIES WITH ALPHA INTERFERON DERIVED FROM HUMAN LYMPHOBLASTOID CELLS, N.B. Finter, G. Allen, K.H. Fantes, M.D. Johnston, T. Priestman, J. Toy and J.G. Woodrooffe, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, England.

We have devised procedures for making interferon on an industrial scale from human lymphoblastoid cells of the Namalwa line. The interferon is highly purified in line during the process. Biochemical analysis has shown that the routine final product issued for clinical use has a mean purity of 82%, and contains eight distinct alpha interferons which differ in their physical, chemical, antigenic and biological properties.

Toxicological and Phase 1 studies have been carried out with preparations of this interferon and clinical trials are now in progress in the U.K., U.S.A., Canada and Japan in patients with various forms of cancer and other diseases.

The mixture of alpha interferons from this source provides a long term alternative to the individual interferons which can be derived from bacteria by recombinant DNA techniques. For clinical purposes the choice will ultimately depend on their relative performance in different medical conditions.

0250 DIRECT INTRAARTERIAL TUMOR INFUSION OF HUMAN INTERFERON-α, M.J. Hawkins, E.C. Borden, J.A. McBain, K.M. Sielaff, and K. Cantell, University of Wisconsin Clinical Cancer Center, Madison, WI 53792 and Central Public Health Laboratory, Helsinki, Finland.

Experience with interferon in animals and chemotherapeutic agents in human neoplasms predicts increased antitumor effect when high intratumor levels of interferon are achieved. Twenty-four patients with metastatic malignant melanoma, colon cancer or breast cancer metastatic to liver (18 patients) or lymph nodes or skin (6 patients) have received direct intraarterial (i.a.) tumor injection of 3-30 x 10^5 units of interferon-α (buffy coat) daily for up to 28 days following an initial i.m. and i.v. injection. Direct intravascular administration of interferon-α was well tolerated clinically. No hepatitis, severe systemic reactions or dose-limiting toxicities occurred. The total amount of interferon appearing in the systemic circulation within 120 minutes of the injection (IFN_{120}) was significantly less after i.a. administration than after i.v. This is consistent with first pass binding of interferon-α to normal and/or cancerous tissue. Peak interferon titers occurred 1 minute after the injection was completed. Secondary, but lower, peaks occurred 15-30 minutes following both i.a. and i.v. administration, consistent with subsequent release of interferon-α from cell membranes. IFN_{120} rose significantly following daily i.a. dosing despite low pre-injection interferon titers. In vitro NK cell stimulation with interferon predicted in vivo activation in 8/9 patients. Enhanced NK cell cytotoxicity was greatest following i.m. and i.v. interferon-α administration and did not change predictably following repeated i.a. injections. Antitumor activity has been observed in patients with melanoma. As a result, the trial continues using escalating doses of interferon-α in patients with melanoma.

Clinical Trial Results II — Antitumor

Sponsored by Cetus/Shell Interferons Program

0251 PHASE I TRIALS OF RECOMBINANT AND NONRECOMBINANT ALPHA INTERFERONS IN PATIENTS WITH DISSEMINATED CANCER. S. Sherwin, S. Fein, J. Whisnant, R. Oldham. Biological Response Modifiers Program, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701; Hoffmann-La Roche, Inc., Nutley, NJ 07110; Burroughs-Wellcome Co., Research Triangle Park, NC 27709.

Approximately 70 patients with a variety of hematologic and non-hematologic malignancies have been treated in Phase I trials of Recombinant Leukocyte A Interferon (IFL-rA) and Human Lymphoblastoid Interferon (HLI) (Wellferon). IFL-rA, which is prepared using recombinant DNA methodology by Hoffmann-La Roche, Inc., represents a single molecular species (clone A) of alpha interferon and has a specific activity of 2 X 10^8 units/mg (>98% pure). In contrast, HLI is prepared from superinduced Namalva cells by Burroughs-Wellcome Co. and is a purified mixture of alpha interferons with a specific activity of 5 X 10^7 units/mg (70% pure). IFL-rA was administered to patients by the intramuscular route at the same dose 3 X weekly for 28 days. 5 or more patients were treated at each of the following dose levels: 1, 3, 9, 18, 36, 50, 68, 86, 100, 118 x 10^6 units. In contrast, HLI was administered to patients by the intravenous route and with escalating doses for each patient. Patients received 5 consecutive treatment cycles consisting of 5 daily 6 hour infusions at the following doses: 0.1, 1, 10, 30, 50 X 10^6 units daily X 5 days. Despite the differences in the properties of these two highly pruified interferon prenarations and the routes and schedules of administration the toxicities observed were similar. Patients experienced fever, chills, fatigue, anorexia, myalgias and less frequently nausea, vomiting and headache. Dose dependent leukopenia was common but corrected quickly when treatment was discontinued. Transient hepatic transaminase elevations were seen only at high doses. Patients were extensively monitored for serum interferon activity and with a panel of immunologic assays and this data will be presented in detail. Of the 42 patients who have completed treatment thus far with IFL-rA, a total of 11 patients with both hematologic and non-hematologic malignancies have demonstrated objective tumor regressions (7 partial remissions + 4 < partial remissions). Of the 8 evaluable patients treated thus far with HLI, 1 patient with an undifferentiated carcinoma has achieved a partial response. Therefore both purified nonrecombinant and recombinant alpha interferons have antitumor activity in vivo.

0252 HUMAN LEUKOCYTE INTERFERON (HuαIFN) IN MULTIPLE MYELOMA (MM): THE AMERICAN CANCER SOCIETY (ACS) SPONSORED TRIAL, E.F. Osserman and W.H. Sherman (Columbia Univ., New York, NY 10032) R. Alexanian and J.U. Gutterman (M.D. Anderson Hosp., Houston, TX) R.L. Humphrey (Johns Hopkins Hosp., Baltimore, MD)

HuαIFN was purchased by the ACS from the Finnish Red Cross. Eight different lots of HuαIFN were employed in different cases. Lots I and III were supplied in the lyophilized state; other lots were not lyophilized. Protocol I: Eighteen cases were previously untreated; 3 cases were in relapse after responding to prior chemotherapy. HuαIFN therapy was initiated with $3x10^6$ U daily: if significant granulocytopenia (<2,000) was not achieved after one month, the dose was increased to $6x10^6$ U daily. Treatment was continued for 6 months unless the disease was clearly progressive. Protocol II: HuαIFN $3x10^6$ U daily for 3 months was added to ongoing chemotherapy in patients in partial remission. Three cases have been entered into this protocol.

Protocol I produced objective evidence of disease regression in only 4 of 21 cases. In these cases, there was a 20 to 70% reduction in the monoclonal protein (MP) and a significant decrease in skeletal symptoms. In 3 cases there was an increase in hemoglobin. These effects were maximal at 4 to 6 weeks and, with one exception, there was no further improvement for the remainder of the period of interferon therapy. There was transient pyrexia and moderate leukopenia in all cases. One case developed pancytopenia, hemolytic anemia and abnormal liver function tests. Protocol II has thus far been instituted in 3 cases. The first case had received HuαIFN under Protocol I for a period of 2 months during which there was evidence of disease progression. She subsequently received continuous low-dose cyclophosphamide (CP) for 12 months and experienced a partial remission. During this period of relative remission, HuαIFN $3x10^6$ U daily was resumed and there was prompt worsening of all disease parameters with reappearance of BJ, increase in serum MP and increasing anemia. the second case was in partial remission on continuous low-dose CP for 4½ years. Addition of HuαIFN $3x10^6$ U daily to CP 75-100 mg daily produced a transient decrease (20%) in serum MP, but exacerbation of anemia. The third case was in partial remission on continuous low-dose melphalan (3 years). Addition of HuαIFN $3x10^6$ U daily produced an abrupt increase in anemia, leukopenia and thrombopenia, but no significant decrease in MP.

The discrepancies between the present results and those previously published are difficult to explain. It is possible that different batches of HuαIFN contained different amounts of the different αIFNs although equivalent concentrations of total, assayable interferon activity. Another alternative which must be considered is that the putative anti-myeloma component in HuαIFN is, in fact, not interferon per se but some other as yet unidentified constituent. Obviously, this latter possibility will be excluded if pure αIFN produced by recombinant DNA technique proves effective in MM.

0253 HUMAN ALPHA INTERFERON TRIALS IN HUMAN TUMOR DISEASES, Hans Strander, Radiumhemmet, Karolinska Hospital, 104 01 Stockholm 60, Sweden.

Human IFN-alpha is being used at the Karolinska Hospital in clinical trials involving: osteosarcoma, juvenile laryngeal papilloma, multiple myeloma, prostate carcinoma, ovarial carcinoma, hypernephroma, malignant melanoma and glioma patients. In most of these studies the standard dose injected is 3×10^6 units intramuscularly daily or 3 times weekly. In some of the studies (myeloma, prostate carcinoma, hypernephroma, malignant melanoma and glioma) are we escalating the dose up to as much as 48×10^6 units daily. Side effects have consisted mostly of fever, shivers and fatique. Immunological parameters have been followed on injected patients and in some cases also the agar colony plaque technique has been employed to test the sensitivity of the tumor cells to IFN. Studies are also being planned for nude mice.

The clinical results indicate that human interferon alpha can exert an antitumor effect in man. Evaluations of the extent of the effect achieved and the efficacy as compared to other modalities of tumor treatment have to rely on clinical trials which so far have not reached a stage where firm conclusions can be drawn concerning persistant clinical results.

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Structure and Mechanisms of Interferon Actions

0254 ACTIVITY OF HYBRID INTERFERON PROTEINS AND THE EXPRESSION OF HUMAN IFN GENES IN EUKARYOTIC CELLS, R. Lawn, A. Franke, C. Houck, A. Levinson and D. Goeddel, Genentech, Inc., South San Francisco, CA 94080

Hybrid human α interferon (IFNα) genes have been constructed and expressed in E. coli by rejoining various IFNα gene segments at common restriction endonuclease cleavage sites. The use of a cell free transcription-translation system has allowed the calculation of specific activities of these hybrid gene products. The hybrid interferons vary in their antiviral activity on different mammalian cells and have activities distinct from their parent molecules.

The viral induction of interferon gene expression has been explored by inserting human interferon genes and their flanking sequences into various non-human eukaryotic cells. The appearance of human IFNα mRNA in these cells has been correlated with various IFNα genes and with portions of their flanking sequences.

0255 FURTHER HETEROGENEITY OF HUMAN α AND β INTERFERON mRNA SPECIES. Pravinkumar B. Sehgal, The Rockefeller University, New York, NY 10021.

Several investigators have recently cloned and characterized at least 8 distinct cross-hybridizing species of IFN-α cDNA and a set of upto 16 cross-hybridizing IFN-α genes and pseudogenes. These are collectively referred to by us as IFN-α_S. We have recently described a second heterodisperse set of translationally active IFN-α mRNAs of length 1.6-3.5 kb (peak activity at 1.8 kb) designated IFN-α_L which code for IFNs which are serologically of the α type but which do not appear to cross-hybridize IFN-α_S-specific DNA probes. Furthermore, analyses of human IFN-β mRNA preparations obtained from poly(I).poly(C)-induced diploid fibroblasts and from several similarly induced human-mouse somatic cell hybrids have led to the detection of at least 5 translationally active human IFN-β mRNA species. The results obtained are consistent with the assignment of 4 of the 5 IFN-β species to human chromosomes 2, 5 and 9. Marked cell-dependent variability in the expression of these IFN-β mRNA species was observed.

0256 HETEROGENEITY IN THE METHYLATED SITES ADJACENT TO HUMAN INTERFERON GENES. Charles H. Riggin, Jr., N. Babu K. Raj and Paula M. Pitha-Rowe. The Johns Hopkins Oncology Ctr, Baltimore, Maryland 21205.

Human DNA from cultured cells and tissue was analyzed by the restriction endonucleases MspI and HpaII for the presence of methylated sites in the sequences flanking the α and β interferon genes. The majority of the DNAs analyzed were methylated in the restriction site (CCGG) for these two enzymes and thus were sensitive to cleavage by MspI, but resistant to HpaII. Three different patterns of methylation were found surrounding the β gene depending upon cell type and tissue analyzed. The β-interferon gene patterns consisted of 1) a single 2.7 kilobase pair (Kbp) fragment, 2) a single 4.4 Kbp fragment, and 3) a combination of both. Induction of β-interferon with poly rI.rC did not alter the methylation pattern in sequences flanking the β-interferon gene. Most of the sites surrounding the α interferon genes also appear to be methylated and therefore, resistant to restriction with HpaII.

0257 THE EFFECTS OF MITOCHONDRIAL METABOLIC INHIBITORS OF INTERFERON ACTION, William J. Richtsmeier, Sidney E. Grossberg, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

The expression of interferon antiviral action was inhibited in human and mouse cells treated with antimetabolites affecting mitochondrial functions. We confirmed earlier observations that cycloheximide, a specific inhibitor of cytoplasmic protein synthesis, failed to inhibit interferon action completely. Chloramphenicol, an inhibitor of mitochondrial protein synthesis, suppressed interferon effect when present in high concentration. In human foreskin cells, the inhibitory effect of 500 μg/ml of chloramphenicol of interferon action (which caused only a 20% inhibition of global cellular protein synthesis) was greater than that observed with 25 μg/ml cycloheximide (which caused 98% inhibition of global cellular protein synthesis); thus, cycloheximide and chloramphenicol together produced a greater inhibitory effect than either alone. A similar observation was made in L-cells. Treatment with cycloheximide in combination with oligomycin, an inhibitor of oxidative phosphorylation, again demonstrated an additive effect. However, no effect was observed either with dinitrophenol, an uncoupler of oxidative phosphorlation, or with ethidium bromide, another respiratory inhibitor. It appears that intact mitochondrial functions are required for the full expression of interferon's antiviral action.

0258 GENETIC ANALYSIS OF THE ROLE OF cAMP IN MEDIATING EFFECTS OF INTERFERON. Jonathan Schneck, Bracha Rager-Zisman, Ora M. Rosen and Barry R. Bloom, Albert Einstein College of Medicine, Bronx, New York 10461.

The effects of interferon (IFN) on Fc-receptor mediated phagocytosis, intracellular cAMP levels, antiviral activity and growth inhibition were analyzed using a cloned macrophage-like cell line, J774.2, and variants derived from it. Purified IFN increased Fc-receptor mediated phagocytosis in J774.2 cells, and in cAMP-responsive nonphagocytic variants, but was without effect in cAMP-unresponsive nonphagocytic variants, in adenylate cyclase variants and in cAMP-dependent protein kinase-deficient variants. Under conditions in which IFN augmented phagocytosis, it increased intracellular levels of cAMP.

Parental cells were highly sensitive to IFN-mediated growth inhibition. In contrast, cAMP-dependent protein kinase variants were 100-fold less sensitive to growth inhibition by IFN.

All cell lines tested, both responsive and unresponsive to cAMP, were equally protected by IFN against infection with vesicular stomatitis virus, demonstrating that the antiviral state was independent of cAMP. These results indicate that in transformed macrophages stimulation of phagocytosis and inhibition of growth by IFN are mediated through intracellular cAMP, whereas the antiviral state induced by IFN is independent of cAMP.

0259 SYNERGISTIC EFFECT OF INTERFERON AND TUNICAMYCIN TREATMENT ON ANTIVIRAL ACTIVITY, Radha K. Maheshwari*, Beena Bhatia** and Robert Friedman*, *Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, **Laboratory of Experimental Pathology, NIADKK, NIH, Bethesda, MD 20505

We have studied the combined effect of tunicamycin (TM) and mouse interferon (IFN) treatment on the antiviral activity against vesicular stomatitis (VSV), Sindbis and encephalomyocarditis (EMC) viruses in mouse L-cells. TM treatment of cells enhance the antiviral activity of IFN significantly against VSV and sindbis viruses; however, no synergistic effect of TM was seen in cells treated with IFN and infected with EMC virus. Similar synergistic effect on antiviral activity against Sindbis virus was observed in chick cells using chick interferon. We have also studied the effect of TM and IFN treatment on VSV proteins and show that both glycoprotein (G) and membrane protein (M) are significantly reduced in VSV released from cells treated with TM and IFN. The synergistic effect on antiviral activity and on inhibition of VSV G and M proteins was observed even with a dose of TM which do not apparently inhibit the cellular or viral glycosylation, thus, suggesting that TM and IFN act through an entirely different mechanism. However, induced changes, that have been reported to occur in the membrane of IFN treated cells and TM, may account for the potentiating effect of TM and IFN against VSV and Sindbis viruses, since these viruses bud from the cell surface as a terminal step.

0260 **HUMAN INTERFERON: BINDING TO AND DEGREDATION BY A HUMAN CELL LINE, Andrew A. Branca and Corrado Baglioni, State university of New York at Albany, Albany New York 12222**

Binding of ^{125}I-labelled human interferon (^{125}I-Hu-IFN-αA) to specific receptors on Daudi cells was demonstrated by the ability of unlabelled Type I human interferons (Hu-IFN-ω, Hu-IFN-β) to compete for the binding of ^{125}I-Hu-IFN-αA. The association of the label with the cells was characterized by equilibrium and saturation binding kinetics. Competition binding experiments with a variety of Type I human interferon preparations indicated that these interferons bind to the same receptor with relatively equivalent affinities. Unlabelled Type II human interferon (Hu-IFN-γ) or mouse interferon (Mu-IFN-β) did not compete significantly for the binding of 125I-Hu-IFNαA indicating that Types I and II human interferons do not share the same receptor and reflecting the known species specificity of Mu-IFN-β .

The association of ^{125}I- Hu-IFN-αA with the cells exhibited a marked temperature dependence. Evidence from competition displacement experiments and characterization of the label released from the cells indicated the possible internalization of interferon and demonstrated directly its degredation by the cells. Correlations of label associated with preparations of cytoskeletal structures implicated a possible role for the cytoskeleton in the processing of interferon after initial binding to cell-surface receptors.

0261 Effects of Human IFN-α on the Biosynthesis of Primate Oncornavirus Proteins John Reynolds, William Stanley and Sandra Panem
The University of Chicago, Chicago, Illinois 60637

Human foreskin fibroblasts productivity infected with simian sarcoma-simian associated virus [SiSV(SSAV)] were examined for viral protein biosynthesis in the presence or absence of human IFN-α. Cantell type IFN-α (Pasteur Institute) or synthetic IFN-α_2 produced in E. Coli (provided by C. Weissmann, Universitat Zurich) was employed. De novo viral protein biosynthesis was examined by SDS-PAGE analysis of immunoprecipitates formed between ^{3}H-leucine labeled cell extracts and monospecific antisera to the major internal viral protein, p30 or the major viral envelope glycoprotein gp70. The patterns of p30-related proteins were the same in interferon treated and untreated cells, whereas patterns of gp70-related proteins differed. The data are consistent with the hypothesis that interferon affects the correct processing of viral envelope proteins.

0262 ANTIRETROVIRAL EFFECT OF INTERFERON: PROPOSED MECHANISM, Robert B. Naso, Yeong-huei C. Wu, and Cheryl A. Edbauer, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Interferon (IFN) treatment of NIH Swiss mouse embryo cells chronically infected with Rauscher murine leukemia virus drastically reduced the release of virus particles from the cells. The characterization of intracellular and extracellular viral specific proteins and polyproteins immunologically with various antisera, and structurally by tryptic digest mapping experiments, indicated that the antiretroviral action of IFN was not due to an IFN-induced alteration in the synthesis of any viral protein. Steady state labeling experiments, however, showed that processing of three viral specific precursor polyproteins, namely gPr90env, Pr40gag, and Pr 25gag, were perceptively slowed in IFN-treated cells. This effect was apparently not related to the ability of these proteins to be modified by phosphorylation or glycosylation after translation since these processes occurred normally in the IFN-treated cells. The treatment of cells with IFN also caused the accumulation of a small amount of a fucosylated viral glycoprotein precursor, termed gP93env, in virus. With the exception of this minor protein, virus released from IFN-treated cells were normal in their content of viral proteins. These virus were only slightly less infectious, particle for particle, than virus released from control cells. Based on these and other results, we suggest that IFN causes an as yet unelucidated alteration in cell membrane structure or function, or both, which prevents recruitment or clustering of an assembly dependant precursor polyprotein, termed Pr65gag, into virus assembly centers in the membrane. Results which may link this mechanism of IFN action to certain IFN-related effects on cell growth and the immune system will also be presented.

0263 ANTIRETROVIAL ACTIVITIES OF γ-INTERFERONS, Ganes Sen, Ruth Herz and Berish Rubin, Sloan-Kettering Cancer Center, New York, NY 10021

γ-IFN is known to have a different spectrum of antiviral activities as compared to α-IFN. No information is available regarding action of γ-IFN on replication of RNA tumor viruses. We tested the antiretroviral activities of human γ-IFN using a human rhabdomyosarcoma cell line, RD-114, which is chronically infected with an endogenous feline retrovirus. γ-IFN was produced by stimulation of human peripheral blood leucocytes with Staphylococcal enterotoxin A and partially purified to a specific activity of 10^5 to 10^6 units/mg protein. The preparations used in these experiments were free of any α or β IFN activity. RD-114 virus production was measured either by measuring the level of virion-associated reverse transcriptase activity or by metabolically labeling the virus using [^{3}H] uridine or [^{3}H] lucine as precursors. Inclusion of increasing doses of γ-IFN in the culture medium resulted in increasing levels of inhibition of RD-114 virus production by these cells. γ-IFN was as effective as α or β IFN in this respect. This indicates that the anti-RD-114 potency of γ-IFN is similar to its anti-VSV potency. The kinetics of establishment of the antiviral state as measured by inhibition of RD-114 virus production was similar for α, β and γ-IFN. Inhibition of virus production was apparent within 3 hr of treatment with γ-IFN and the maximum level of inhibtion was reached by 12 hr. Even at the highest dose of γ-IFN used (200 units/ml) there was no appreciable inhibition of total cellular RNA or protein synthesis.

0264 REVERSAL OF THE 2-5 OLIGO A DEPENDENT INHIBITION OF PROTEIN SYNTHESIS IN INTACT CELLS WITH OLIGONUCLEOTIDES, Roberta J. Black[1], Robert M. Friedman[2], Jiro Imai[3], and Paul F. Torrence[3], Laboratories of Experimental Pathology[1] and Chemistry[3] NIADDK, NIH, Bethesda, Md. 20205 and Department of Pathology[2], Uniformed Services University of the Health Sciences, Bethesda, Md.

The oligoribonucleotide, p5'A2'p5'A2'p5'A, has been shown to be capable of preventing the protein synthesis inhibitory properties of 2-5 oligo A in cell-free extracts of mouse L cells. Such an antagonist of 2-5 oligo A could be valuable in ascertaining the role of 2-5 oligo A in mediating the antiviral effects of interferon if such a molecule could be introduced into an intact cell. For this purpose, we have explored the use of the calcium phosphate coprecipitation technique of Graham and van der Eb to transfect 2-5 oligo A and its antagonist into intact mouse L cells. The triphosphorylated trimer, pppA2'p5'A2'p5'A, caused maximum inhibition at a concentration of 10^{-6}M using a calcium concentration of 75mM or 100mM. Similarly, transfection of the tetramer triphosphate, pppA2'p5'A2'p5'A2'p5'A, resulted in maximum inhibition of protein synthesis at a concentration of 10^{-6}M. Using identical conditions, the antagonist was transfected at a concentration of 10^{-4}M simultaneously with the trimer triphosphate at a concentration of 10^{-6}M resulting in a complete reversal of the inhibition of protein synthesis. The specificity of this antagonistic action has been examined using several analogues and derivatives of trimer core monophosphate.

0265 CHEMICAL MODIFICATION POTENTIATES THE BIOLOGICAL ACTIVITY OF 2-5A AND ITS DERIVATIVES.
Jiro Imai, Margaret I. Johnston and Paul F. Torrence, Laboratory of Chemistry, NIADDK, NIH, Bethesda, Md. 20205

Chemical modification of the 2' terminus of 2-5A tetramer core 5'-monophosphate to give 5'-O-monophosphoryladenylyl(2'→5')adenylyl (2'→5')adenylyl (2'→6')(3'-aza-N-hexyl-2',3', 4'-trideoxyglucopyranos-1'-yl)adenine resulted in a 2-5A antagonist which was 10 times more potent than 2-5A tetramer core 5'-monophosphate itself. When this same modification was carried out at the level of the 5'-triphosphate, the resultant analogue was 10 times more potent than 2-5A as an inhibitor of protein synthesis in cell-free extracts of mouse L cells with an IC_{50} of 7.10^{-11}-1.10^{-10} M compared to an IC_{50} of 1.10^{-9} M for 2-5A. As judged by degradation of radioactive EMCV RNA under protein synthesis conditions, this increased potency for inhibition of protein synthesis was matched by an increase in potency for the activation of the 2-5A-dependent endoribonuclease. The half-life of this new analog was compared to that of 2-5A by two separate methods: persistence of biological activity and persistence of reactivity towards antibody directed against 2-5A. Under conditions of protein synthesis, 2-5A possessed a half-life of about 20 minutes whereas the terminally modified analog was essentially undegraded after 2.5 hours.

0266 ANTAGONISM OF THE ACTION OF 2-5A BY 2-5A CORE MONOPHOSPHATE: STRUCTURE-ACTIVITY RELATIONSHIPS, Krystyna Lesiak, Jiro Imai and Paul F. Torrence, Laboratory of Chemistry, NIADDK, NIH, Bethesda, Md. 20205

Information regarding the structural features which are important in binding of oligoribonucleotides to the 2-5A-dependent endoribonuclease has been obtained through study of the ability of various chemically modified 2-5A core monophosphate analogues to antagonize the protein synthesis inhibitory effects of 2-5A. Methylation at N-6 or bromine substitution at C-8 of the purine ring, viz, p5'm^6A2'p5'm^6A2'p5'm^6A and p5' br^8A2'p5'br^8A2'p5'br^8A, respectively, resulted in ≥ 100-fold decrease, relative to p5'A2'p5'A2'p5'A, in ability to prevent the translational inhibition caused by 2-5A. The 2'(3')terminally phosphorylated p5'A2'p5'-A2'p5'A2'p was ∿ 10 times more potent than 2-5A core monophosphate as an antagonist. Although the dimer p5'A2'p5'A was without antagonistic activity, 2'(3')terminal phophorylation gave p5'A2'p5'A2'p which was only 10X less active than core monophosphate. Phosphodiester linkage isomers showed the following order of activity p5'A2'p5'A3'p5'A < p5'A3'p5'A2'p5'A < pA2'p5'A3'p5'A2'p5'A < pA3'p5'A2'p5'A2'p5'A < trimer or tetramer core monophosphate. In summary, these data suggest: a) that binding to the 2-5A activated endoribonuclease is more sensitive to changes in the adenine rings than to changes in the phosphate-sugar backbone, b) that loss of the 2' terminal adenosine caused a significant, but by no means total loss of binding, and c) that the endonuclease will tolerate an oligoribonucleotide in which at least one of the phosphodiester bonds is 3'5'.

0267 MEASUREMENT OF 2'5' OLIGOADENYLATE SYNTHETASE IN HUMAN MONONUCLEAR CELLS,
J. A. Merritt, E. C. Borden, L. A. Ball, Departments of Human Oncology, Biochemistry, and the Biophysics Lab, University of Wisconsin, Madison, WI 53792

Interferon treatment of cells induces the synthesis of 2'5' oligoadenylate (2'5'A) synthetase. In order to examine the potential of 2'5'A synthetase as a clinical method by which to monitor interferon therapy, we standardized an assay which measured the enzyme activity in cytoplasmic extracts of Ficoll-purified human peripheral mononuclear cells (PMC). The 2'5'A synthetase in the extracts was bound to and activated by poly(I).poly(C) agarose beads. The assay quantitated the incorporation of [^{3}H]ATP into phosphatase-resistant oligoadenylate "cores" that were bound to and eluted from DEAE filters. The assay was sufficiently sensitive to measure basal and induced levels of enzyme activity in 10^6 normal human PMC. One unit of activity incorporated 1 pmole ATP per hour at 37°C. The reaction rate was linear for at least 20 hours and, within limits, the amount of product was proportional to the amount of enzyme assayed. Treatment of PMC in culture with IFN-∝ produced a graded increase in enzyme activity up to at least 100 units of interferon per ml. PMC from healthy volunteers had enzyme levels of 28 ± 24 (S.D.) units per 10^6 cells. Increased activity was found in individuals who reported recent viral syndromes, as well as in patients with metastatic breast cancer. Moreover, enzyme activity in PMC from the latter group of patients increased further during daily injections with IFN-∝. Serial samples from patients treated on the American Cancer Society phase II breast cancer study are currently being analyzed.

0268 ANTIVIRAL MECHANISM OF INTERFERON: ACTIVATION OF INTERFERON-INDUCED PROTEIN KINASE IN MOUSE L929 CELLS BY REOVIRUS INFECTION, Sohan L. Gupta and Sandra L. Holmes, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

We have tested whether the interferon (IFN)-induced protein kinase is activated upon virus infection which may be required if it plays a role in the IFN-induced antiviral mechanism. In earlier studies, no activation of this kinase could be observed upon infection of IFN-treated mouse L929 cells with either vesicular stomatitis virus or mengovirus. Here we report that reovirus infection results in an activation of this enzyme. This is indicated by (i) the phosphorylation in vivo of a 67,000 Mr (67K) polypeptide, which is characteristic of IFN-induced protein kinase, upon reovirus infection of IFN-treated mouse L929 cells, and (ii) the phosphorylation in vitro of the 67K polypeptide as well as the 38K α-subunit of exogenously added initiation factor eIF-2 in the extracts of IFN-treated reovirus-infected cells without the addition of double-stranded RNA which is required in similar extracts from uninfected cells. This activation of protein kinase is observed within a few hrs after reovirus infection and provides the first example of in vivo activation of IFN-induced protein kinase upon virus infection. Reovirus cores isolated from infected cells could activate this protein kinase in extracts as tested either by the phosphorylation of the 67K polypeptide or the 38K α-subunit of eIF-2. Addition of reovirus cores to a cell-free translation system from IFN-treated cells strongly inhibited the translation of mengo mRNA. Under the conditions where the kinase activation was observed, little or no activation of 2',5'-oligoadenylate synthetase could be detected.

0269 MEASUREMENT OF 2-5A AND RELATED OLIGONUCLEOTIDES IN SV40-INFECTED, INTERFERON-TREATED CELLS, Carol Lipsey Hersh and George R. Stark, Stanford University, Stanford, CA 94305

Treatment of cells with interferon leads to increased levels of 2-5A synthetase which catalyzes the formation of pppA2'(p5'A)$_n$ (2-5A) from ATP in the presence of ds RNA. However, 2-5A levels have been measured in only a few systems. We have developed a new radioimmunoassay which has enabled us to measure subnanomolar concentrations of 2-5A and other oligonucleotides of related structure, using antibody made against pppA2'(p5'A)$_3$ linked to bovine serum albumin. Our assay complements the endonuclease-based radiobinding assay developed by Ian Kerr and coworkers which has different specificity for compounds related to 2-5A. In general, the radioimmunoassay detects two classes of compounds not detected by the radiobinding assay: compounds with only a single terminal 2'-linked AMP residue and 2-5A oligomers with one or no 5' phosphoryl group. For example, we can detect the oligonucleotide NADpA which is synthesized in vitro by 2-5A synthetase from NAD and ATP. With the radioimmunoassay we have observed 2-5A - like material in CV1 cells infected with SV40 and treated with interferon 8 to 24 hours later. This material is present at substantially lower levels in cells only treated with interferon or only infected with SV40. The concentration of 2-5A - like material measured by the radioimmunoassay is about ten times greater than that measured by the radiobinding assay. Analysis of extracts, from SV40-infected, interferon-treated cells, which were fractionated by high pressure liquid chromatography indicates that 2-5A and A2'(p5'A)$_n$ are present but account for a minority of the material we observe. Studies on the formation and structure of the 2-5A - like material will be presented.

Immune Relationships and Biology of Interferon

0270 STUDIES OF T CELL CLONES AND THE ROLE OF INTERFERON IN EFFECTOR FUNCTION AND REGULATION. M.A. Cooley, Dept of Biological Sciences, University of Warwick 4CV 7AL U.K

Clone 3.1 has been obtained by in vivo sensitization and in vitro restimulation of CBA lymp node cells to trinitrophenyl-(TNP) modified syngeneic stimulating cells. After cloning by limiting dilution, clone 3.1 was found to (a) have an absolute requirement for T cell growth factor (TCGF) ; (b) to proliferate additionally in response to presentation of H-2K^k + TNP, (c) to be specifically cytotoxic for H-2K^k TNP, but to kill only LPS-induced blast cells, not Con A induced blasts or tumor cells (RDM4) which present H-2K^k; (d) to specifically suppress the induction of H-2^k TNP specific CTL in vitro. A subclone, 3.1.24, retained the cytotoxic capacity of the parent, but had lost suppressive capability. The role of interferon in the suppression/cytotoxic effector functions of this and other T cell clones, and in the regulation of interactions of such immunocompetant cells is the object of my present studies.

0271 THE DISSOCIATION OF THE ANTIVIRAL AND IMMUNOMODULATING ACTIVITIES OF THE HUMAN INTERFERONS BY OXYPHENBUTAZONE, William M. Mitchell, Robert L. Forti, James T. Forbes, Robert Workman and Walter Hubbard, Depts. of Pathology, Medicine and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232

Among the pleiotropic effects of human interferon (HuIFN) are the inhibition of viral replication and the activation of natural killer (NK) cells. Oxyphenbutazone, a non-steroidal anti-inflammatory agent, is a potent inhibitor of the antiviral activity of HuIFN-α and HuIFN-β as determined by cytopathic effect and virus (VSV) synthesis and release in human foreskin fibroblasts (SG-181). The inhibition of IFN activity is dose dependent with maximal inhibition at 50 μM and minimal inhibition at 5-10 μM. The drug has no irreversible direct effect on IFN and has no apparent cellular toxicity at the concentration used. In contrast, oxyphenbutazone, at concentrations as high as 100 μM has no effect on the activation of NK cells by HuIFN. The major prostaglandin species, as determined by HPLC of ^{3}H-arachadonic acid metabolites, synthesized by SG-181 cells in the presence of IFN and VSV are 6-keto-$PGF_{1\alpha}$ and PGE_2 while human lymphocytes and contaminating blood cells isolated on Hypaque-Ficoll synthesize $PGF_{2\alpha}$, thromboxane B_2 (i.e. from platelets) and PGE_2. Radioimmunoassay and GC/MS were used to quantitate the inhibition of these prostaglandin species in SG-181 cells by inhibitors of fatty acid cyclooxygenase. Aspirin, indomethacin, phenbutazone, and oxyphenbutazone at 10 μM and higher concentrations effectively inhibit the accumulation of 6-keto-$PGF_{1\alpha}$ and PGE_2. However, aspirin, indomethacin, and phenbutazone have no inhibitory effect on IFN antiviral activity. Studies are in progress to similarly quantitate the effect of cyclooxygenase inhibitors on arachadonic acid metabolites in the NK cell system and to extend these observations to the antiproliferative properties of IFN. (Supported in part by ACS Institutional Research Grant IN-25U and USPHS Grant No. CA 23477.)

0272 RELATIONSHIP BETWEEN SURFACE ANTIGEN DENSITY AND NK-ACTIVITY OF HUMAN PERIPHERAL BLOOD LYMPHOCYTES WITH OR WITHOUT ADDITION OF α-INTERFERON. M. Hokland, I. Heron, P. Hokland and K. Berg. Institute of Medical Microbiology, University of Aarhus, and University Department of Haematology and Medicine, Aarhus Amtssygehus, Denmark.

FACS sorting of human peripheral blood lymphocytes into four fractions according to size (scatter) and surface content of β_2-Microglobulin (β_2-M) or HLA molecules (fluorescence intensity) has enabled us to investigate the relationship between NK-activity and the surface density of the above mentioned antigens. Generally, large lymphocytes exhibited the highest NK activity, and likewise, lymphocytes with high amounts of surface β_2-M molecules were better NK effectors than those with low β_2-M content. However, since small lymphocytes with high β_2-M content were better NK effectors than large lymphocytes with low β_2-M content considerable heterogeneity was found. Importantly, the NK cells that could be boosted by α-interferon were found solely in the high antigen-density fractions. Interestingly, the close relationship between surface antigen density and NK activity thus demonstrated for β_2-M could not be found for HLA molecules (defined with an anti-framework-HLA monoclonal antibody) indicating a dissociation between these two otherwise closely related antigens.

Further data elucidating the role of β_2-M in the IFN-enhancement of NK will be presented.

0273 INTERFERON ENHANCES THE ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES. P. Hokland, M. Hokland and K. Berg. University Department of Haematology and Medicine, Aarhus Amtssygehus and the Institute of Medical Microbiology, University of Aarhus, Aarhus, Denmark.

Polymorphonuclear leukocytes (PMN) from healthy volunteers were tested for ADCC activity against both erythrocyte and tumor targets with and without the addition of human leukocyte interferon (IFN). It was demonstrated that IFN within 3o to 6o min enhanced the reaction in a dose-dependent manner with minimal IFN doses ranging from 1 to 1oo units. Formal proof that the augmenting agent was IFN was obtained by using pure IFN proteins in combination with both mock-IFN preparations, which showed no enhancing activity, and anti-IFN antisera, which inhibited the action of the completely purified IFN proteins. In the light of data demonstrating that the IFN effect was most pronounced when the IgG antibodies in the ADCC reaction were present in suboptimal amounts, it is hypothesized that IFN may play a special role in the early nonspecific immune response against non-self antigens.

0274 IMMUNE INTERFERON MODULATION OF INTERLEUKIN 2 RECEPTORS, William L. Farrar, Henry C. Stevenson and Howard M. Johnson, Frederick Cancer Research Facility, Frederick, MD 21701 and The University of Texas Medical Branch, Galveston, TX 77550.
Activation of T lymphocytes requires both antigen and immune interferon (IFNγ) to acquire a state of proliferative responsiveness to Interleukin 2(IL2). In both murine and human T cell activation systems, pretreatment of purified T cells with IFNγ or Concanavalin A (ConA) activated cells to become responsive to the growth promoting activity of IL2. Both IFNγ and lectin activation was neutralized by the addition of anti-IFN sera in both murine and human systems. Furthermore, physiochemical destruction of IFNγ by pH2.0 dialysis also abrogated the ability of the IFNγ preparation to activate T cells, susbstantiating the activating principle as IFNγ. The acquired proliferative responsiveness to IL2 by IFNγ pretreated T cells was shown to be a function of the biological expression of IL2 receptors. The data demonstrate the requirement of IFNγ in the modulation of activation processes which lead to the development of growth factor receptors on cell surfaces.

0275 INTERFERON SYNTHESIS BY HUMAN THYMOCYTES AND T-LYMPHOCYTES, Gabrielle H. Reem, Laura Cook, Dorothy Henriksen, and Jan Vilček, New York University Medical Center, New York, N.Y. 10016
T-lymphocytes synthesize gamma interferon when cultured with B lymphoblastoid cells or with either Concanavalin A or PHA. Coculture of T-lymphocytes with B lymphoblastoid cells and with Concanavalin A or PHA does not further augment interferon synthesis. Thymocytes produce only small amounts of interferon when stimulated by the lectins Concanavalin A or PHA or by B lymphoblastoid cells. However, the joint stimulus of B lymphoblastoid cells and one of the lectins results in a synergistic effect, and interferon synthesis exceeds that of lectin stimulated thymocytes more than one hundred fold. Both mature and immature thymocytes can be induced to synthesize interferon. Interferon synthesized by stimulated thymocytes or T-lymphocytes is not neutralized by antiserum to alpha or beta interferon; it is unstable at pH 2 and is therefore most likely gamma interferon. In contrast, interferon produced by B lymphocyte lines is neutralized by alpha interferon and stable at pH 2. Pretreatment of thymocytes or T-lymphocytes with 12-O-tetradecanoylphorbol-13-acetate (TPA) does not stimulate interferon synthesis by thymocytes, but does induce interferon synthesis by T-lymphocytes. However, co-stimulation of thymocytes with TPA and either Concanavalin A or PHA induces high levels of interferon synthesis. These studies indicate that thymocytes induced by two stimuli can produce significant amounts of interferon.

0276 SYNTHESIS OF HUMAN γ-INTERFERON IN YEAST, Rik M. Derynck, Arjun Singh, Frank E. Hagie, Ronald A. Hitzeman and David V. Goeddel, Genentech, Inc., South San Francisco, CA 94080

A cDNA coding for human immune (γ) interferon was incorporated into hybrid plasmids which are able to be propagated both in *E. coli* and in the yeast, *Saccharomyces cerevisiae*. After introduction of these plasmids into yeast, the sequence coding for the mature polypeptide is expressed under the control of a yeast promoter giving rise to biologically active γ-interferon molecules.

0277 STRUCTURE OF THE IMMUNE INTERFERON GENE, Patrick W. Gray and David V. Goeddel, Genentech, Inc., South San Francisco, CA 94080
A cDNA coding for human immune interferon (IFN-γ) has recently been identified from a cDNA colony library (see Goeddel *et al.*, these abstracts). This immune interferon cDNA has been utilized as a probe in Southern hybridizations of human genomic DNA to demonstrate that only a single gene codes for immune interferon. In contrast to leukocyte interferon (IFN-α) and fibroblast interferon (IFN-β), the immune interferon gene contains at least one intervening sequence. The immune interferon cDNA has also been used to identify recombinant λ clones containing human immune interferon gene sequences. This gene sequence will be presented.

0278 IMMUNOMODULATION BY IFN-CONJUGATED MONOCLONAL ANTIBODY TO HUMAN OSTEOGENIC SARCOMA
Graham R. Flannery, Julie M. Pelham, J. Dixon Gray and Robert W. Baldwin, Cancer Research Campaign Laboratories, University Park, Nottingham NG7 2RD, U.K.

Purified human lymphoblastoid interferon-α (IFN-α) has been coupled covalently, using N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), to a mouse monoclonal antibody raised against the human osteogenic sarcoma cell line 791T. This antibody has been described elsewhere (Embleton et al, Brit. J. Cancer, 43, 582, 1981) and binds specifically to cells of the immunizing tumour and to a number of other osteogenic sarcoma cell lines. The conjugate was purified by Sephacryl S-200 gel filtration in order to remove free IFN and double-radiolabelling studies indicate a final IFN:antibody molar ratio of 0.5:1. The purified conjugate retains both antibody activity and IFN activity:binding to 791T cells can be shown in competitive binding assays using fluorescent-labelled antibody and the Fluorescence Activated Cell Sorter (FACS IV), and human peripheral blood lymphocyte-mediated lysis of 791T cells and the erythroleukaemic cell line K562,is significantly enhanced after 1 hr incubation with effector cells. The antibody has been shown to localize in 791T xenografts in immunodeficient mice. The conjugate has been tested in an in vitro model system: in the presence of IFN-antibody conjugate bound to 791T cells, human NK cells exhibit significantly enhanced killing of a second, radiolabelled target (K562). Pre-incubation of mixtures of IFN and antibody with 791T cells failed to produce NK cell stimulation. This reagent represents a novel and potentially effective therapeutic tool which may allow specific targetting of IFN to the tumour site in vivo and subsequent activation of tumour-infiltrating NK cells.

0279 INDUCIBILITY OF HUMAN LUPUS INCLUSIONS BY INTERFERONS, Steven A. Rich and Timothy R. OWENS, Div. Labs. and Res., N.Y.S. Department of Health, Albany, NY 12201

Human lupus inclusions (LI) are an abnormal anastomosing network of microtubular inclusions which ultramorphologically mimic paramyxovirus. Type I interferons (IF) were recently shown (Science, 213, 772, 1981) to stimulate a B human lymphoblastoid cell line (HLCL), Raji, to form LI. Seventeen HLCL formed 4 distinct categories of LI frequencies: 21%, 11%, 2% and 0% when stimulated for 70 hrs by 100 units of human lymphoblastoid IF/ml (K.C. Zoon). Daudi, the only line in the 21% category, plateaued at an LI frequency of 15% at 10 units of IF/ml and increased to 21% LI at 50 units/ml. Raji, the only line in the 11% category, plateaued at an LI frequency of 11% at 50 units/ml. IF inhibited the growth of Daudi but not Raji. Luk II (L.H. Kronenberg) did not contain LI, even though Daudi formed LI when grown in conditioned medium which contained 256 units of IF/ml spontaneously secreted by Luk II. Additional results suggested specificity for the formation of LI: 1) mouse type I IF (R.M. Friedman) neither induced LI in Raji nor in a B mouse LCL, S49A2, whose growth was severely inhibited at 10 units/ml; 2) human fibroblast IF at 100 units/ml induces LI in Raji, protects GM2504 and WISH from VSV infection at 1 unit/ml but does not stimulate the formation of LI in either GM2504 or WISH even at 200 units/ml; 3) human type II IF (Y.K. Yip) at 5 and 50 units/ml neither induced LI nor inhibited the growth of Raji or Daudi nor affected their response to type I IF. Changes in the titer of IF by the HLCL could not account for the formation of LI or growth inhibition. Our findings with HLCL may provide analogies for results to be expected with cells from patients with "spontaneous" elevated levels of IF or who are on IF therapy. (Supported by the American Heart Association, Northeastern New York Chapter, Inc.)

0280 EFFECT OF HUMAN LEUKOCYTE INTERFERON ON COLLAGEN SYNTHESIS BY CULTURED HUMAN FIBROBLASTS, Joel Rosenbloom, Dental School, U. of Pa., Philadelphia, Pa. 19104

The human leukocyte interferons represent a family of proteins that exhibit a number of biological activities. In order to begin studies on the capacity of interferon to modulate the behavior of resident cells of the connective tissue, crude leukocyte interferon or pure human recombinant leukocyte interferon (designated IFL and IFLrA respectively, generous gifts of Dr. Sidney Pestka, Roche Inst. of Molecular Biology) were tested for their ability to modulate collagen synthesis by normal human dermal fibroblasts. Contact-inhibited, confluent cultures were studied in order to avoid effects on growth and the fibroblasts were incubated in Eagles medium containing 5% fetal calf serum, 50 μg/ml ascorbate, [^{14}C]proline and various interferon concentrations for 48-72 hr. Collagen synthesis was estimated by [^{14}C]hydroxyproline analysis, collagenase digestion and gel filtration chromatography. The crude interferon produced a dose-dependent inhibition of collagen synthesis with 50% inhibition at 500 units/ml and 65% inhibition at 2000 units/ml. General protein synthesis was only minimally inhibited. Although inhibition of collagen synthesis was also observed with the recombinant interferon, higher concentrations, 5,000 units/ml, were required to produce a 35% inhibition. Neither interferon preparation produced any effect on fibroblast viability as measured by trypan blue exclusion. Leukocyte infiltration is a frequent response in a variety of circumstances and these results suggest that some forms of interferon may play a physiologic role in regulating the activities of connective tissue cells. Supported by NIH grants AM-20863, AM-20553 and DE-02623.

0281 DIFFERENTIATION OF HUMAN MYELOID OR MONOCYTE CELL LINES. INTERFERON AS WELL AS PHORBOL ESTERS PROMOTE DIFFERENTIATION TO A MONOCYTE-MACROPHAGE PHENOTYPE, Michael Pack, Toshio Hattori, Zong-liang Chang, Randy Zicht, and Thomas Hoffman. Laboratory of Immunodiagnosis, National Cancer Institute, Bethesda, MD 20205.
A series of myelomonocytic cell lines were examined under conditions known to promote differentiation to more mature forms. The criteria of differentiation used were morphology, the ability to produce superoxide, surface markers, and acquisition of lytic activity. Dimethyl sulfoxide (1%), Actinomycin D (5μg/ml), or hypoxanthine (10 μg/ml) added to the culture medium resulted in differentiation of HL60, but not other myeloid (KG-1, K562) cell lines to more mature granulocyte forms. Phorbol myristate acetate (PMA, 10 ng/ml) treatment promoted differentitation of HL60 and the monocyte line U937 to cells with the morphology and adherence characteristics of macrophages. U937 cells differentiated by PMA also demonstrated enhanced expression of the OKM1 antigen and manifested antibody-dependent cell cytotoxicity (ADCC) against chicken red blood cells. Treatment of U937, but not HL60, with 400 units/ml of human β-interferon similarly resulted in differentiation to functional macrophages, with a slightly delayed time course. These studies illustrate that pathways of differentiation inherent to a given cell line may be evoked by a variety of dissimilar agents, and indicate a need to consider interferon's ability to promote differentiation among the range of its diverse biological activities.

0282 DEVELOPMENTAL CONTROL OF INTERFERON PRODUCTION IN THE SYRIAN HAMSTER, Paul O.P. Ts'o, James J. Greene and Robert Dyer, Biophysics Division, The Johns Hopkins University, Baltimore, Md. 21205
Induction of interferon (IFN) during embryogenesis of the Syrian hamster has been characterized with respect to (1) the subpopulation of IFN-producing cells and (2) the molecular structure of the excreted IFN. Cell cultures derived from embryos excised at 9 through 13 days gestation were induced to synthesize IFN by exposure to U.V.-inactivated N.D.V. The subpopulation of IFN-producing cells was examined by an agar-overlay "zone of protection" as well as by antiviral activities exhibited by a defined number of N.D.V. treated cells through serial dilutions. Using these assays, cultures established from 9-day gestation (9-dg) embryos contained ten times the number of cells producing IFN than 13-day gestation (13-dg) cultures. In vitro passaging of the 9-dg cells reduced the number of zones to the level of the 13-dg cells while passaging had no effect on 13-dg cells. 9-dg cultures produced at least two kinds of IFN based on antiviral activities: a non-binding fraction on blue Dextran Sepharose columns (BDSC) at pH 6.4 with apparent molecular weights (mol.wt.) of 20-22 K as determined by gel filtration and a binding fraction to BDSC with a mol.wt. of 26-27 K. From 13-dg culture, antiviral activity was found only on the non-binding fraction to BDSC with a mol.wt. of 19-21 K. Thus, the IFN(s) produced from Syrian hamster embryos and the subpopulation of cells producing IFN are greatly influenced by the developmental stage of embryogenesis. (Supported by N.I.H. grant CA 27120)

0283 INHIBITION OF DIMETHYLBENZ(a)ANTHRACENE INDUCED MUTAGENESIS BY INTERFERON. John J. Reiners, Jr., Larry P. Yotti, Thomas J. Slaga, Univ. of Tennessee School of Biomedical Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.

An in vitro cell-mediated mutagenesis assay was used to analyze the effects of interferon on polycyclic aromatic hydrocarbon (PAH) dependent mutagenesis. Newborn SENCAR murine keratinocytes were used for the metabolic activation of the promutagen 7, 12-dimethylbenz(a) anthracene (DMBA), and Chinese hamster lung V-79 fibroblasts were used for detection of the resulting mutagens. Mutations at, or affecting the hypoxanthine-guanine phosphoribosyltransferase locus were scored by resistance to 6-thioguanine. A dose-response relationship was observed for DMBA concentration and mutant recovery. Preincubation of the keratinocytes with 500 units/ml of murine fibroblast B-interferon 26 and 6h prior to the addition of a toxic (1-4% survival) and mutagenic dose (1μM) of DMBA, resulted in 71 and 47% reductions, respectively, in the mutation frequency. Interferon was less effective if added simultaneously with DMBA (34% inhibition) and ineffective if added 4-14 h after DMBA. The extent of inhibition was dose dependent over an interferon range of 10-500 units/ml. Concentrations of interferon $\leq$ 500 units/ml were neither toxic to the keratinocytes or V-79 cells nor enhanced the rate of keratinocyte terminal differentiation. These data suggest that B interferons prevent or alter keratinocyte metabolic activation/detoxification of the aromatic hydrocarbon, DMBA.

0284 EFFECT OF INTERFERON ALPHA ON POKEWEED MITOGEN-INDUCED DIFFERENTIATION OF HUMAN PERIPHERAL BLOOD B LYMPHOCYTES, Yong Sung Choi, Sloan-Kettering Institute for Cancer Research, Rye, New York 10580.

The effect of human leukocyte interferon (IFN-α) on pokeweed mitogen (PWM)-triggered differentiation of human B cells has been studied *in vitro*. Graded doses of IFN-α were added to the culture of human peripheral blood monocytes, and total antibody synthesis was measured after six to seven days of culture by using a highly sensitive reverse hemolytic plaque assay. The results suggest that a low dose (10^2 U/ml) enhances and a high dose (10^4 U/ml) suppresses B-cell differentiation. Its action occurred in an early stage of B-cell differentiation. Since PWM-triggered differentiation of human B cells requires T cells and monocytes in the culture, we have examined separately the effects of IFN-α on T cells and monocytes. The data indicate that enhancement was mediated by activation of monocytes and suppression was due to inhibition of helper T-cell proliferation. At a high dose, B-cell proliferation may be also directly inhibited by IFN. The implication of these findings is that IFN is involved directly in immunoregulation during viral infection.

0285 [THE BASIC FEATURE OF NK-IFN SYSTEM AND POSSIBLE INVOLVEMENTS IN HUMAN DISEASES.] N.Minato, Jichi Medical School, Tochigi, JAPAN
Increasing evidences indicate that NK cells do play significant roles in host defence mechanisms against viral infections and tumors. Using a unique experimental system, we have indicated, (1) NK cells are primarily responsible for the rejection of RNA virus persistently infected tumor cells in T cell-deficient nude mice (J.Exp.Med. 149:1117.1979), (2) IFN is a major factor regulating NK activity in which IFN induces recruitment of pre-NK cells associated with characteristic changes of effector phenotypes (J.Exp. Med. 150:124.1980), (3) this IFN-mediated NK regulation is a essential process in in vivo control of the tumor cells in nude mice (Proc.Natl.Acad. Sci. 78:1171.1981), and (4) NK effector can be heterogenous in terms of phenotypes, genetics, and regulations, each of which may have distinct roles in host defence system (J.Exp.Med. in press). Based on these findings, we further investigated the functions of NK-IFN system in patients with Sjögren syndrome who have extremely high incidences of lymphomas and those with subacute sclerosing panencepharitis which is a typical disease caused by persistent virus infection in human. The results clearly indicated the dysfunctions of NK-IFN system in these patients (J.C.I. in press). These analysis would open a new therapeutical approach including IFN in these diseases where no effective therapy is available yet.

Clinically Predictive and Animal Studies

0286 LYSIS OF FRESH ACUTE LEUKEMIA CELLS BY INTERFERON-ACTIVATED HUMAN NATURAL KILLER(NK) CELLS, Paul K. Pattengale, Alice Yu, Christer Sundstrom, and Hans Wigzell, USC School of Medicine, Los Angeles, CA 90033, UC San Diego, San Diego, CA 92103, and the University of Uppsala, Uppsala, SWEDEN S751-23.

Fresh, purified, noncultured leukemic cells from four patients with chronic granulocytic leukemia(CGL) in blast crisis, three patients with T-cell acute lymphocytic leukemia(T-ALL), and nine patients with common childhood acute lymphocytic leukemia (cALL) were found to be susceptible to lysis by human natural killer (NK) cells obtained from healthy donors. This NK susceptibility was substantially increased when effector peripheral blood lymphoid cells(PBLs) were activated with uncloned human leukocyte interferon(HLIF) preparations.

Target	Mean Lytic Units per 10^7 PBLs		Mean % Increase with HLIF
	-HLIF	+HLIF	
T-ALL	3.4	18.9	555.9
cALL	5.6	28.9	516.1
CGL in blast crisis	8.4	43.8	521.4
K-562	56.4	284.3	504.1

Although less susceptible than the standard NK target K-562, the observed lysis of the fresh, noncultured,neoplastic leukemic cells was mediated by a population of interferon-augmentable FcR positive, nonadherent lymphoid cells, which were also able to kill the standard NK target K-562 as measured by cold target inhibition assays. The clinical implications of these findings will be presented in relation to the neoplastic and nonneoplastic targets of NK lysis.

0287 ANTI PROLIFERATIVE ACTIVITIES OF INTERFERON, M.C. Troxell, R. Rice, N. O. Kaplan and E. Grunberg, University of California, San Diego, La Jolla, California 92093

We have previously reported that different interferons have different anti-growth and anti-viral activities[1]. We have now found that interferons have remarkable effects on cells in culture when they reach the stationary stage and that some interferons can completely kill all cells when they are in this stage. The inhibition of cells in the growth phase is considerably less when compared to the confluent cells. This phenomenon has been observed on three human tumor cell lines: colon, astrocytoma, and prostate. Recombinant leukocyte interferon A (IFLrA) produced in E. coli was determined to have specific activities of 1.7×10^8 anti-viral (AV) units/mg protein and 4×10^5 anti-growth (AG) units/mg (AV/AG=4.25×10^4). Crude CML interferon had activities of 4×10^4 AV units/mg and 50 AG units/mg (AV/AG = 800). Thus the AV/AG ratio for IFLrA is approximately 50 fold higher than that for the crude CML interferon. Nevertheless, IFLrA inhibits cell growth in culture at levels of 0.01 µg/ml. Furthermore, we have found that IFLrA can slow the growth of human tumors in athymic mice when given at doses of 0.02-0.1 µg/mouse/day. We conclude from this study that IFLrA definitely possesses anti-growth activity.

[1] N. O. Kaplan and S. Slimmer, Cellular Responses to Molecular Modulators, Proc. 1981 Miami Winter Symp., Vol 18, pp. 443-453, Eds. L. W. Mozes, J. Schultz, W. A. Scott and R. Werner (Acad. Press) 1981.

We thank Hoffmann-La Roche Inc. and NIH (CA-11683) for support.

0288 BIOLOGICAL PROPERTIES OF GENETIC HYBRIDS OF BACTERIA-DERIVED HUMAN LEUKOCYTE INTERFERONS, Phillip K. Weck, Sharon Apperson, Elizabeth Hamilton, and Nowell Stebbing, Genentech, Inc., South San Francisco, California 94080

The presence of common restriction endonuclease sites in the gene sequences of human leukocyte interferons cloned in bacterial systems has facilitated the production of hybrid interferon molecules consisting of the amino terminus of one interferon and the carboxy terminus from another. A series of sixteen different hybrids and LeIF-I, -J, and -K have been screened for antiviral activity in vitro on five different cell types challenged with Vesicular Stomatitis (VS) or Encephalomyocarditis (EMC) virus. Two of these hybrid molecules LeIF-AD (Bgl II) and LeIF-AD (Pvu II) have high specific activities on human Hela, mouse L-929 and bovine MDBK cells. In vivo experiments demonstrated that highly purified preparations of these interferons are effective in protecting mice, hamsters and squirrel monkeys against lethal doses of EMC virus. A third hybrid LeIF-AI generated by splicing between amino acids 151 and 152 has characteristics similar to the two LeIF-AD hybrids indicating that the terminal amino acids of the interferon molecule are important for biological activity. Comparative studies utilizing bacteria-derived human hybrid leukocyte, fibroblast and gamma interferons will lend added insight to the species specificity of these interferons and determine whether new and more potent materials can be generated by recombinant DNA technology.

0289 IMMUNOLOGICAL MONITORING OF PATIENTS RECEIVING RECOMBINANT LEUKOCYTE A INTERFERON. A.E.Maluish[1], J.R.Ortaldo[2], S. Sherwin[2], R. Leavitt[3], D.M.Strong[4], S. Fine[5], P.Wiernik[3], R.K.Oldham[2] and R.B.Herberman[2]. USUHS, Bethesda; BRMP NCI-FCRF,Frederick; BCRC, Baltimore; NMRI, Bethesda; Hoffman-La-Roche, Nutley, N.J.

NK activity, monocyte function, lymphoproliferative responses and enumeration of lymphocyte subpopulations using monoclonal antibodies and analysis by flow cytometry were performed on 100 patients receiving recombinant leukocyte A interferon (IFN). Patients were treated in 2 phase-1 studies to determine the maximum tolerated dose (to be presented separately) and the optimum immunomodulatory dose. IFN was given by intra-muscular injection either twice daily or three times weekly for 28 days with groups of 5 patients receiving one of several doses of IFN. Doses ranged from 0.5 - 100 million units. Blood specimens were obtained 2-3 times prior to treatment to determine the baseline reactivity in each assay and then 7-8 times during the course of treatment. NK activity did not show any significant or sustained elevation at any dose of either protocol. In fact, there was a trend towards a depression of NK activity. The possible reasons for this difference from previously reported studies will be discussed. Monocyte function, as measured by a growth inhibition assay, did show a sustained elevation in many patients. Preliminary determinations of lymphoproliferative responses show a decrease in some patients and enumeration of lymphocyte subpopulations show an increase in OK T10 positive cells in some patients. The correlations of these data with the functional data will be discussed.

0290 Phase I Trial of Human Leukocyte Interferon in Patients with Advanced Cancer.
J.R. Neefe, T. Sreevalsan, M. Harris, F.P. Smith, and J.A. Bash. Lombardi Cancer Research Center, Georgetown University, Washington, D.C. 20007

25 patients (pts) with advanced malignancy were treated with human leukocyte interferon (IFN). Pts in groups of 3-5 received a single dose of IFN ($0.5\text{-}60\times10^6$ units/m^2). Toxicities included universal fever and chills, headache, myalgias, and fatigue. Nausea and vomiting, leukopenia or thrombocytopenia, hematuria, and transaminasemia were transient and infrequent. Symptomatic hypotension was observed in 1 severely anemic pt. Immunomodulatory effects on 3 activities were tested: natural killing (NK), antibody-dependent cellular cytotoxicity (ADCC), and monocyte-mediated cytotoxicity (MMC). 3 pretreatment observations and post-treatment observations on days 1, 3, and 7 were normalized with a standard frozen control. A modest decrease in NK and ADCC was sometimes seen at d. 1. Two pts had significant increases in NK and ADCC at d. 3; one of these, with breast cancer, had stable disease. One pt had a striking decrease in NK and ADCC; his rhabdomyosarcoma continued its previous pattern of rapid progression. A few pts had increased MMC at d. 3. There was no clear correlation of IFN level with changes in NK or ADCC. One partial response was seen in a pt with previously untreated melanoma and an abdominal mass. A single dose of human leukocyte interferon up to 60×10^6 units/m^2 does not produce consistent augmentation of NK, ADCC, or MMC, but can produce objective tumor regression. Supported by NIH Contract NCI-CM-07437-1.

0291 Abstract withdrawn

0292 LIPOSOMAL - INTERFERON: ALTERED PHYSICO - CHEMICAL AND PHARMACOLOGIC PROPERTIES WITH POTENTIAL FOR CLINICAL APPLICATIONS.
Deborah A. Eppstein, Syntex Research, Palo Alto, CA 94304

Association and properties of human and mouse interferons (IFN) (alpha and beta) with liposomes was studied, employing different physical and chemical composition liposomes. Depending on the liposome composition, IFN could be retained from one to three days in the vicinity of the local injection site. In contrast, equivalent doses of free IFN resulted in undetectable local IFN levels one day after injection. Liposome compositions could be chosen that resulted in apparent incorporation of IFN within the liposomal lipid bilayer, such that the IFN was protected from trypsin digestion, yet was able to interact with cells without prior disruption of the liposome. Use of such IFN - liposome preparations is being tested in animal models for increased ability to induce tumor regression after intralesional application. This mode of administration may be particularly useful for β IFN. Intravenous injection of liposomal - IFN also resulted in altered pharmacologic properties of the IFN, with increased retention/accumulation of IFN in the lungs, spleen, and liver as compared to levels obtained with free IFN. Interferon administered in this manner is being tested for its ability to modulate various immune parameters, and for its ability to combat metastatic tumors in mice.

0293 SEQUENTIAL ADMINISTRATION OF POLYRIBOINOSINIC ACID (ssI) AND POLYRIBOCYTODYLIC ACID (ssC) INDUCES INTERFERON (IF) AND DEPRESSES HEPATIC P-450 DRUG METABOLIZING SYSTEMS
Gilbert J. Mannering and Laurel B. Deloria, Department of Pharmacology, University of Minnesota, School of Medicine, Minneapolis, Minnesota 55455

Poly rI·rC induces IF and depresses hepatic P-450-linked drug metabolizing systems. Neither ssI nor ssC are effective inducers of IF or depressors of P-450 systems. However, DeClercq and DeSomer (Science 173, 260: 1971) showed that when ssI and ssC are administered sequentially in that order and the time between administrations is within 10 min, IF is induced in mice. No IF was induced when the order of administrations was reversed. In the current study, mice were injected (i.p.) with ssI (5 mg/kg) followed 1 hr later with ssC (5 mg/kg). The mice were killed 22 hr after the second injection and the P-450 content and ethylmorphine (EM) N-demethylase activity of hepatic microsomes were measured. P-450 was depressed 30% and EM N-demethylase 40%; neither effect was seen when the order of injections was reversed. Similar results were observed when the interval between injections was 5 min or 2 hr, but the depressions were not significant when the interval was 4 or 8 hr. Two, 6 and 12 hr after the sequential administration of ssI and ssC, the serum was shown to contain 1050, 1206 and 1370 units of IF/ml; at corresponding time intervals the livers contained 70, 95 and 70 units of IF/g. Corresponding values obtained from mice which received ssC followed by ssI were serum: 50, 25 and 25 and liver: 25, 21 and 15 units of IF/g. These studies support previous evidence that IF depresses hepatic P-450 systems. Supported by USPHS Grant GM 15477.

0294 INTERFERON PRODUCTION IN MULTIPLE SCLEROSIS PATIENTS, Aimo Salmi, Reijo Salonen, Mauri Reunanen and Jorma Ilonen, University of Turku and University of Oulu, Finland. Peripheral blood lymphocytes from multiple sclerosis (MS) patients and age- and sex-matched healthy blood donors were stimulated with PHA, PWM and PPD, which preferentially stimulate production of gamma-interferon (IFn-γ). Measles, rubella, mumps and herpes simplex virus antigens were used to stimulate alpha-interferon (IFN-α) production. A standard plaque reduction assay with vesicular stomatitis virus (VSV) was used to measure IFn levels in lymphocyte culture supernatants.

MS patients in a stable phase of the disease produced less interferon than matched controls with all the stimulants. The number of individuals without any detectable interferon was significantly greater in the MS patients than in the controls. The lower response seems to be related to the Dw2 histocompatibility antigen. Since no correlation between the reduced IFn production and any clinical variables was seen this phenomenon may indicate a primary weakness in immune regulation of these patients.

Because the standard plaque reduction techniques is not sensitive enough for very small amounts of IFn, a new technique based on measurement of released VSV was developed for IFn measurements. This technique is more accurate than the standard technique and is 4 to 8 times more sensitive.

0295 INTERFERON AND INTERFERON INDUCED ENZYMES IN NEWBORN CALVES. John Wéregne[1], C.Van den Broecke[1], M.Verhaegen[2], L.Dagenais[3], A.Schwers[3], E.Thiry[3] and P.P.Pastoret[3]; 1.Université Libre de Bruxelles, Fac. des Sciences; 2. Institut Pasteur du Brabant; 3.Laboratoire de Virologie, Faculté de médecine vétérinaire, Université de Liège.

Experimental infection of colostrum deprived newborn calves with different strains of bovine Rotavirus, obtained from cell cultures (4th to 6th passage after isolation from stools of calves dead from severe diarrhoea) causes interferon production. The detection of interferon in the serum is indicative of virus replication which causes later on a characteristic seroconversion. Such an experimental system is therefore suitable for the investigation of the possible usefulness of the determination of the activity of the interferon induced enzymes for the clinical detection of a viral infection in cattle.

Furthermore, as we also showed that both human and bovine interferons induce the 2'5'oligoadenylate-synthetase in bovine cells, this model may be used to ascribe the efficiency of different interferon preparations for antiviral therapy.

0296 A MONOCLONAL ANTIBODY-BASED IMMUNORADIOMETRIC ASSAY FOR HUMAN LEUCOCYTE INTERFERON IN SERUM, Jane Hewitt', Jackie R. Walker[2], J. Nagington[2], Stephen R. Abbott' and David S. Secher[3]; Celltech Ltd, Slough', Public Health Laboratory, Addenbrooke's Hospital[2] and MRC Laboratory of Molecular Biology[3], Cambridge, U.K..

An immunoradiometric assay (IRMA) originally developed for monitoring the purification of leucocyte interferon (IFNα) (Secher, Nature 290 501, 1981), has been significantly modified and optimised so that it may now be applied to the measurement of IFNα in human serum. The assay is a 'sandwich'-type IRMA, in which IFNα binding to a solid-phase polyvalent sheep anti-IFN antibody is determined using a radioiodinated monoclonal antibody (^{125}I-NK$_2$) to HuIFNα. The assay developed is both sensitive (detection limit ≅ 10U/ml) and reproducible (inter-assay CV <20% over the linear dose-response range, 50-10^4 U/ml) and may conveniently be performed within 24 hours. We have applied the assay to the measurement of IFNα in 250 normal sera. Of these, two had significantly elevated values. Using sera from 9 volunteers who received intramuscular HuIFNα, a significant correlation was observed between serum IFN values determined by IRMA and those obtained using a biological assay (inhibition of viral nucleic acid synthesis). Our results suggest that the monoclonal-based IRMA provides a convenient and reliable method for the rapid estimation of circulating IFNα in man as an aid for monitoring treatment.

0297 IN VIVO PROPERTIES OF MURINE INTERFERON-ALPHA AND -BETA, Jan Trapman, Arnold Hekman and Eric Prins, Department of Pathology, Erasmus University, Rotterdam, The Netherlands.

Murine interferon-alpha (Mu IFN-α) and murine interferon-beta (Mu IFN-β) were tested for their properties as an anti-viral and anti-tumor agent in a murine experimental model: Rauscher murine leukemia virus-induced erythroleukemia in BALB/c mice. Mouse interferon was produced by poly(I).poly(C)-induced L-929 cells and separated into the Mu IFN-α and Mu IFN-β component by a combination of gel filtration and anti-interferon affinity chromatography. Interferon treatment was started eight to ten days after virus inoculation and was continued for another three days. Thereafter, mice were sacrificed and the effect of interferon on the development of the leukemic process was monitored by measuring the spleen weight, histological examination of the spleen, investigation of the presence of virus-infected cells in the spleen by immunofluorescence techniques using an anti-gp70 antibody, determination of reverse transcriptase activity in the serum and measuring of the hematocrit. Our data show that both Mu IFN-α and Mu IFN-β are able to stop the further development of the leukemia in this three-day period. Application of high doses ($6\text{-}10\times10^4$ U/day) of Mu IFN-α even results in a diminution of the amount of virus-infected cells in the spleen. Because the same dosis of Mu IFN-β does not give rise to a similar decrease in spleen weight, our data indicate that Mu IFN-α is more effective as an anti-virus/tumor agent in this model system than Mu IFN-β.

0298 ROLE OF INTERFERON PRODUCTION AND ACTION IN NATURAL RESISTANCE OF INBRED MICE TO HERPES SIMPLEX VIRUS TYPE 1 (HSV-1). R. Zawatzky, I. Gresser+, E. DeMaeyer* and H. Kirchner, Institute of Virus Research, German Cancer Research Center, Heidelberg, +Institut de Recherches Scientifiques sur le Cancer, Villejuif, and *Institut Curie Orsay France.

HSV-1 is a neurotropic virus that causes lethal encephalitis in several strains of mice 1 to 2 weeks after intraperitoneal (i.p.) infection. C57BL/6 (B6) mice are relatively resistant to infection with HSV 1 whereas other strains such as DBA/2 or BALB/c are quite susceptible. In this study we report the paradoxical finding that B6 mice were resistant to 250 lethal doses$_{50}$ (LD_{50}) of HSV-1 (= 10^6 PFU) when injected i.p , but that they were killed by 25 or 2.5 LD_{50}. In contrast in DBA/2 mice no resistance to any virus dose higher than 2.5 LD_{50} could be observed. Measurement of interferon titers at the injection site showed that in B6 mice 10^6 PFU (= 250 LD_{50}) of virus induced high titers of interferon (> 1000 IU) that reached maximal levels 2-4 hours after infection. In HSV-susceptible DBA/2 mice, however, the same dose of virus induced only about 10 times less interferon in the peritoneal cavity 2-4 hours after i.p. infection. When we decreased the virus dose per animal to 10^5 and 10^4 PFU (25 or 2.5 LD_{50} in B6 mice, respectively) only borderline levels of interferon (15 40 IU) could be detected in peritoneal fluids from B6 mice. By simultaneous inoculation of 250 LD_{50} of HSV-1 and anti-mouse interferon serum, B6 mice were rendered susceptible. However, anti-interferon treatment did not increase susceptibility to one LD_{50} of virus. Two injections of mouse interferon given 2 hrs before plus 2 hours after virus infection protected B6 mice against 25 LD_{50} of virus but had no effect in HSV infection of DBA mice. It is concluded that genetically determined resistance of mice to infections with HSV-1 is influenced by genes not only coding for rapid interferon production but also for the antiviral action of interferon.

0299 STUDIES ON INTERFERON (IFN) PRODUCTION AND HEMATOPOIETIC SUPPRESSION. Philip Paul, Susan Rothmann,Kathleen Sturkie,Marge Kraus,Dale Duca,and Max Proffitt,Cleveland Clinic, Cleveland,OH 44106

Induction of IFN production and hematopoietic inhibition following chronic administration of poly (I:C) is being investigated as a model for IFN-induced myelosuppression which may progress to aplastic anemia. Female 9-12 week old C57Bl/6 mice received poly (I:C) continuously via osmotic minipumps (Alza) implanted in the peritoneal cavity. Doses ranging from 1-5 ug/hr were delivered for 7 day periods. On day 8, plasma IFN, peripheral blood counts, and committed hematopoietic stem cells were measured. 1 ug/hr had no effect on hematology or IFN production. After one week of treatment at 5 ug/hr, significant anemia developed (Hb reduced by 3 gm/dl). IFN averaged 85 U/ml. Marrow erythroid progenitors, CFU-e, were reduced to 17% of control. Spleen CFU-e were markedly increased and appeared to be important for recovery that occurred 1 week after cessation of treatment. No significant changes in early erythroid progenitors, BFU-e, or any granulocyte parameter were observed. The development of anemia may be related to IFN induction or reflect poly (I:C) toxicity, a question currently being addressed by infusion of IFN directly.

0300 ANTIPROLIFERATIVE ACTIONS OF INTERFERON AND DOUBLE-STRANDED RNA: INDUCTION OF RESISTANT VARIANTS IN HUMAN TUMOR CELL POPULATIONS, James J. Greene, Stanley L. Lin, and Paul O. P. Ts'o, Div. of Biophysics, Johns Hopkins Univ., Baltimore, Md. 21205 and William A. Carter, Orlowitz Inst., Hahnemann Medical College, Philadelphia, Pa. 19102.
Human fibroblast interferon (IFN) and the dsRNA, $rI_n \cdot rC_n$ were found to exert a pronounced inhibitory effect on the proliferation of fibrosarcoma(HT1080) and bladder carcinoma(RT4) cells. This inhibitory effect was much less on normal human fibroblasts. Proliferation of HT1080 and RT4 cells was completely arrested at 400 u/ml and 80 u/ml of IFN respectively, while proliferation of normal fibroblasts was only partially inhibited at 400-1000 u/ml IFN. Culture of HT1080 and RT4 cells in IFN-containing medium resulted in the emergence of subpopulations resistant (IFN^r) to the antiproliferative effects of IFN. Luria-Delbruck fluctuation analysis indicated that the IFN^r trait is nonrandomly acquired and is induced by IFN selection with a relatively high (0.1-4%) but varying frequencies within different IFN-sensitive (IFN^s) clonal populations. Preliminary results indicate that the IFN^r trait reverts to IFN^s within 5-10 cell divisions when cultured in the absence of IFN. The IFN^r cells retained sensitivity to the anti viral effects of IFN and were sensitive to the antiproliferative effects of $rI_n \cdot rC_n$ even in the presence of antibodies to IFN. Intracellular 2'-5' oligoadenylate synthetase level in IFN^r-RT4 cells was found to be 1.5 to 3 fold higher than in IFN^s-RT4 cells, whereas no differences were found between IFN^s and IFN^r-HT1080 cells. For IFN^r-RT4 cells, 2 of 4 clones acquired resistance to dsRNA toxicity at frequencies above parental cells. Since IFN^r and $dsRNA^r$ traits can be acquired independently and transiently through a nonmutational mechanism, the antiproliferative actions of IFN and $rI_n \cdot rC_n$ may not be identical. (Supported by N.I.H.).

0301 EFFECT OF DIFFERENT MODES OF POLY (I:C) ADMINISTRATION ON INTERFERON (IFN) INDUCTION, Kathleen Sturkie, Philip Paul,Max Proffitt,and Susan Rothmann, Cleveland Clinic, Cleveland, OH 44106
Attempts to maintain continuously high circulating IFN levels have not been entirely successful. To test whether different administration regimens could affect induction of IFN by synthetic polyribunocleotide, poly (I:C) was given to 9-12 week old female C57Bl/6 mice intraperitoneally as single injections or continuously via an intraperitoneally implanted osmotic minipump (Alza). Mice were bled at various times and the plasma analyzed for IFN using a virus yield-reduction semi-micro assay. 7 hours after a 125 ug injection, 10^4 U/ml IFN were present. Splenectomy immediately prior to injection caused a reduction to 10^3U/ml. At 18 hours after injection, IFN was 10^3U/ml and 10^2U/ml in intact and splenectomized mice respectively, and declined to non-detectable levels rapidly thereafter. When 125 ug was injected daily for 7 days, only a small amount (~10U/ml) was detected in the intact animals and none in the splenectomized mice. A significantly higher amount (40-100 U/ml) was present in animals receiving poly I:C continuously via the minipumps. In all experiments, comparable toxicity was observed with 20% mortality, and significant anemia. Use of an implantable minipump appears to allow sustained induction of IFN at levels higher than single injections. Whether use of IFN in the pumps will give higher circulating levels than daily injection, or with poly I:C induction, is being tested currently.

0302 LYMPHOCYTE RECIRCULATION AFTER TREATMENT WITH INTERFERON OR POLY I:C, K.J. Blank, R. Korngold and D.M. Murasko, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, The Wistar Institute, Philadelphia, PA 19104 and The Medical College of Pennsylvania, Philadelphia, PA 19129
Intravenous inoculation of either partially purified Type I mouse interferon or poly I·poly C, an interferon inducer, results in a 5 to 10 fold reduction in output of thoracic duct lymphocytes (TDL) in mice 8 to 24 hours after treatment. Pretreatment of mice by intraperitoneal inoculation with sheep anti-mouse interferon abolished the effect of both interferon and poly I· poly C. TDL obtained from mice with reduced output were typed for lymphocyte antigens and showed no major alteration in concentration of B or T cell subsets. This interferon-mediated reduction in recirculating lymphocytes is transient since normal levels of TDL are seen 1 to 2 days after treatment. When ^{51}Cr labelled lymph node cells are inoculated i.v. into mice that have received poly I·poly C, there is a significant increase in the number of labelled cells found in the lung. The mechanism and duration of this trapping is being investigated. This decrease in lymphocyte recirculation observed after treatment with or induction of IFN may contribute to the immunosuppression observed when interferon and antigen are inoculated simultaneously.