INTERFERONS AS CELL GROWTH INHIBITORS AND ANTITUMOR FACTORS

Robert Friedman, Thomas Merigan and T. Sreevalsan, Organizers

April 6 — April 12, 1986

Plenary Sessions

April 7:
Interferons as Immunoregulators 215–216
Interferon Action on Human Tumors 217

April 8:
Gene Regulation by Growth Factors and Interferons (Joint) 99–100
Gene Regulation II 218–220

April 9:
Structure-Activity Relationships of Interferons and Interferon Receptors 220–223
Biological Activities of Interferon and Animal Models — I 223–224

April 10:
Biological Activities of Interferon and Animal Models — II 225–227
Pharmacology of the Interferons 228

April 11:
Inhibitors of Growth and Transformation (Joint) 105-106, 229

Poster Sessions

April 7:
Immunoregulation, Receptors and Clinical Studies
(Poster Abstracts M30 — M46) 230–235

April 8:
Interferons and Oncogenes
(Poster Abstracts M47 – M57) 235–239

April 9:
Gene Regulation and Differentiation
(Poster Abstracts M58 – M63) 239–241

April 10:
Pharmacology and Biological Activities
(Poster Abstracts M64 – M80) 241–246

Late Additions

(Poster Abstracts M81 – M84) 247–248

Interferons as Immunoregulators

M0 EFFECTS OF CYTOKINES (IFN-ALPHA, IFN-GAMMA, TNF, AND IL-1) ON MONOCYTE FUNCTIONS, Lois B. Epstein and Ramila Philip, University of California,San Francisco,CA 94143.

The recent availability of pure natural or recombinant DNA produced human lymphokines and monokines and poly or monoclonal antibodies to these pure reagents has made it possible to dissect some of the complex interactions which these mediators of immunity have with each other and with cells of the immune system. Our studies show that, in the presence or absence of LPS (endotoxin), IFN-alpha, IFN-gamma, and TNF enhance the production of IL-1 by human monocytes. Furthermore, these same agents and also IL-1 enhance monocyte cytotoxicity as determined in a ^{51}Cr release assay using actinomycin-D treated WEHI cells as targets. The ability of TNF and IL-1 to enhance cytotoxicity is specific for monocytes, as TNF and IL-1 do not enhance natural killer (NK) cell activity. This is in contrast to IFN-alpha and IFN-gamma where both monocyte and NK cytotoxicity are enhanced. Our studies thus demonstrate that IFN-alpha, IFN-gamma, and TNF share in common the ability to enhance IL-1 production and monocyte cytotoxicity. However, enhanced production of IL-1 is not the mechanism whereby IFN-alpha, IFN-gamma, and TNF enhance monocyte cytotoxicity. Additional experiments in which the effects of IFN-alpha, IFN-gamma and TNF on monocyte cytotoxicity were studied in the presence of neutralizing antibodies to IL-1 revealed that the antibodies to IL-1 did not prevent the enhancement of monocyte cytotoxicity by IFN-alpha, IFN-gamma and TNF. Our results highlight the fact that TNF, like IL-1 and the IFNs, is pleiotropic in its effects. Furthermore, although these agents overlap in the monocyte functions they mediate, the individual mechanisms appear to be distinct.

M1 EFFECTS OF INTERFERONS ON NATURAL KILLER CELL ACTIVITY, ANTIBODY-DEPENDENT CELLULARCYTOTOXICITY AND MACROPHAGE-MEDIATED CYTOTOXICITY, Ronald B. Herberman, Pittsburgh Cancer Institute Pittsburgh, PA 15213.

Most species of interferon alpha or beta (IFNα or β) have been shown to appreciably augment natural killer (NK) cell activity. However, there are substantial quantitative differences among the interferon species in regard to the dose needed for augmentation. Human IFNαJ has been shown to be quite efficient in augmentation, with no effects being observed after short term pre-incubation with effector cells. Recombinant murine or human IFNγ have also been found to have only weak augmenting capacity for NK activity. Antibody-dependent cellular cytotoxicity (ADCC) against tumor target cells, utilizing either polyvalent or monoclonal antibodies, has been shown to be mediated predominantly by large granular lymphocytes (LGL), the same subpopulation responsible for NK activity. IFNα and β have been shown to also substantially augment ADCC activity by LGL. In contrast to the augmenting effects upon pretreatment of effector cells, pretreatment of the target cells often results in decreased susceptibility to subsequent lysis. The ability of the various interferons to induce this protection of target cells differs substantially from the NK-augmenting effects, with IFNγ being substantially more effective than IFNα or β . In regard to augmentation of macrophage-mediated cytotoxicity against tumor cells, IFNγ has been shown to be most potent. Overall, the available results indicate that interferons can have a complex series of modulatory effects on effector cell reactivity against tumor target cells, with the net result depending on the type of interferon utilized, the effector cell involved, and the possible induction of resistance to lysis if the target cells are exposed to interferon prior to the effector cells.

M2 GROWTH FACTORS AS INTERFERON INDUCERS AND INTERFERON INDUCERS AS GROWTH FACTORS. Jan Vilček, Masafumi Tsujimoto, Dorothy Henriksen-DeStefano, Vito J. Palombella, Makoto Hirai and Masayoshi Kohase. New York University Medical Center, New York, N.Y. 10016 and Suntory Institute for Biomedical Research, Osaka, Japan.

Highly purified E. coli-derived recombinant human tumor necrosis factor (TNF) acts as a potent growth factor for human diploid fibroblasts.[1] Stimulation of cell growth was demonstrable at a TNF concentration of 3×10^{-13} M and maximal stimulation was attained at TNF concentrations $\geq 1 \times 10^{-10}$ M. The Kd of TNF binding to receptors in several human cell lines was found to be approximately 2×10^{-10} M.[2,3] We conclude that stimulation of cell growth is probably a physiological function of TNF and that the cytotoxic and cytostatic actions of TNF may be the result of an anomalous growth signal transduction in neoplastic cells lacking the constraints of normal growth control mechanisms.

IFNs are known to inhibit the mitogenic effect of many growth promoting agents, including TNF.[1] We have shown that TNF acts as an inducer of IFN-beta in human FS-4 fibroblasts and that the presence of antibody to human IFN-beta can enhance the mitogenic action of TNF (M. Kohase et al., this Symposium). We now show that the IFN-beta inducer, poly(I)·poly(C) exerts a mitogenic effect in FS-4 cells. Inclusion of antibody to IFN-beta greatly enhanced the mitogenic effect of poly(I)·poly(C). In contrast, the mitogenic effect of epidermal growth factor was not affected by antiserum to IFN-beta. These results corroborate the recent finding that in murine 3T3 cells poly(I)·poly(C) stimulates the expression of the same competence gene family (including IFN-beta) as platelet-derived growth factor.[4] Induction of IFN-beta by some growth factors apparently represents a physiological negative feedback mechanism. The primordial function of IFNs may have been the regulation of cell proliferation. (Supported in part by NIH grant AI-12948 and by a grant from Suntory Limited.)

[1]Vilček, J., et al., J. Exp. Med., in press
[2]Tsujimoto, M., et al., PNAS 82, 7626 (1985)
[3]Tsujimoto, M., et al., J. Immunol., in press
[4]Zullo, J.N. et al., Cell 43, 793 (1985)

M3 ENDOGENOUS AND EXOGENOUS INTERFERON (IFN) SIGNALS IN MACROPHAGE (MØ) DIFFERENTIATION, Stefanie N. Vogel, Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

A fully "activated" MØ reflects the end result of a complex differentiative scheme which involves a specific sequence of environmental or cell-derived differentiative signals. IFNs either endogenously produced or exogenously provided, represent a major class of such MØ differentiative signals.

One approach we have taken to gain insights into the role of endogenous IFN in MØ differentiation is to utilize inbred mouse strains which differ only at the gene which controls responsiveness to endotoxin (LPS). Our previous work demonstrated that peritoneal MØ from mouse strains fully responsive to LPS (Lps^n) continue to differentiate in vitro, as evidenced by an increased capacity to bind and phagocytose via the Fc receptor. In contrast, MØ from LPS-hyporesponsive strains (e.g., C3H/HeJ) exhibit no such increase and lose with time in culture the capacity to bind or phagocytose particles opsonized with IgG. This defect in MØ differentiation was found to be correctable by treatment of cultures with exogenous IFN-α,β, or γ. A comparison of the differentiative capacities of C3H/HeN (Lps^n) and C3H/HeJ (Lps^d) MØ resulted in the following findings: (i) Anti-α/β antibody greatly reduces the Fc receptor capacity of C3H/HeN MØ; (ii) C3H/HeJ MØ can be made more phagocytic by coculture with C3H/HeN MØ or by treatment with supernatants derived from C3H/HeN MØ cultures; and (iii) C3H/HeN MØ spontaneously lose Mac-1 antigen with time in culture. C3H/HeJ MØ must be IFN-treated to be equivalently down-regulated. These observations supported the hypothesis that Lps^n MØ, in contrast to LPS-hyporesponsive MØ, produce higher levels of an autostimulatory differentiation signal that appears to be MØ-derived IFN. This hypothesis has been extended by the finding that C3H/HeJ MØ are clearly much more permissive for VSV infection than are C3H/OuJ (Lps^n) MØ. One can increase permissiveness in Lps^n MØ by treatment of cells with anti-IFN-α/β antibody prior to infection.

Exogenous addition of IFNs to MØ cultures also results in dramatic differentiative changes. Using the C3H/HeJ MØ model, an anti-IFN-γ monoclonal antibody was used to inhibit recombinant IFN-γ-induced antiviral activity, Fc receptor, and Ia antigen expression. Although these three functions are induced over approximately the same dose range, significantly more antibody was required to inhibit Fc induction and antiviral activity than induction of Ia. These findings are consistent with the possibility that there may exist on the IFN-γ molecule functional epitopes. Lastly, in many systems, one observes an additive or synergistic effect of IFN-γ with IFN-α or IFN-β. Treatment of MØ with IFN-γ and IFN-α/β blocks induction of Ia antigen by IFN-γ (IFN-α/β are inactive). Thus, antagonism between these two IFN classes may provide important information with respect to control of IFN-γ-induced differentiation during an inflammatory episode. Supported by Grant #AI18797.

Interferon Action on Human Tumors

M4 Antitumor studies with the interferons in human malignancies. Jordan U. Gutterman, Department of Clinical Immunology and Biological Therapy, U.T. M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

During the last eight years, we have been conducting clinical and laboratory investigations with the interferons in a variety of human malignancies. Both partially pure alpha interferon (Finnish Red Cross) and alpha A interferon (Roferon, Hoffman-LaRoche) have the capacity to induce tumor regression in some solid tumors, such as renal cell carcinoma and to induce significant hematologic remission in chronic leukemias, such as hairy cell leukemia and chronic myelogenous leukemia. Approximately 80 per cent of patients with hairy cell leukemia achieve either partial or complete remission with alpha interferon. Updated analysis of the duration of remission with both partially pure and recombinant alpha A interferon will be presented. Additionally, about 80 per cent of patients with benign phase chronic myelogenous leukemia achieve hematologic remission. Recently, studies with recombinant alpha A interferon have demonstrated that a significant percentage of patients achieve reduction of the percentage of the Philadelphia chromosome-bearing cells in the bone marrow. At least three of eighteen patients in the current study have demonstrated total disappearance of the Philadelphia-bearing cells in the bone marrow. Studies of cell surface receptors for interferon, induction of 2,5'A synthetase, and modulation of the abl oncogene have been carried out serially in the patients treated in vivo with alpha interferon. Currently, our studies suggest that resistance to alpha interferon is due to a defect beyond the presence of the alpha interferon receptor.

More recently, we have been conducting studies with recombinant gamma interferon. In addition to reviewing the pharmacokinetic and biological behavior of gamma interferon, we will illustrate the ability of gamma interferon to induce hypertriglycerdemia and inhibition of the post-heparin lipase activity. Recent studies of combining alpha A interferon and recombinant gamma interferon will be discussed.

Conclusion. The interferons are clearly active antitumor agents. Recent studies and plans to amplify the antitumor activities of interferon in combination with other biological and chemotherapeutic agents will also be discussed.

M5 OVERVIEW OF CLINICAL EFFICACY AND SAFETY OF RECOMBINANT ALFA-2A (ROFERON®-A) IN CANCER PATIENTS, Loretta M. Itri, Hoffmann-La Roche Inc.

Since January 1981, recombinant interferon alfa-2A (Roferon®-A) has been evaluated in clinical trials of more than 1300 patients with a broad spectrum of oncologic disease. Patients with either solid tumors or hematologic malignancies were treated with daily, three-times-weekly, or daily escalating dose intramuscular or subcutaneous injections for induction periods ranging from 8 to 16 weeks. In the daily escalating group, the dose was increased over 12 days from 3×10^6 units to 36×10^6 units. Therapy was continued at the highest dose for the remainder of treatment in the absence of toxicity. Because of the diversity of the patient population and the indications studied, patient entry and exclusion criteria varied to a small degree in different studies, but, overall, the criteria remained consistent throughout. In general, patients were eligible for entry into Roferon®-A clinical trials if they were at least 18 years of age, had histopathologically documented disease with a prognosis for life expectancy greater than the duration of induction, had a performance status of greater than or equal to 60 (Karnofsky scale) or less than or equal to 2 (Eastern Cooperative Oncology Group scale), and required no palliative treatment during the study. Written informed consent was obtained from all patients. Doses ranged from 1×10^6 units to 124×10^6 units per injection. Beneficial clinical effects have been noted in a variety of malignant disorders including hairy cell leukemia, AIDS-related Kaposi's Sarcoma, renal cell carcinoma, cutaneous T-cell lymphomas and other tumor types. The most striking clinical results thus far have been obtained in patients with hairy cell leukemia treated with relatively low daily doses of 3×10^6 units. When administered at this dose, Roferon®-A was well tolerated, and dose attenuation was rarely required. Change to a three-times-weekly treatment regimen at the same dose was usually sufficient to control toxicity when it occurred in this group of low-dose patients. Those patients receiving significantly higher doses frequently required dose attenuation to 50% of the starting dose to improve clinical tolerance. Virtually all patients treated with Roferon®-A experienced some degree of acute toxicity manifested as fever, chills, myalgia, and/or headache. These reactions usually occurred with initial dosing and frequently improved spontaneously with continued administration of the drug. Acetaminophen pretreatment was generally useful in ameliorating these symptoms. Common adverse experiences occurring after repeated dosing included fatigue, anorexia, and weight loss. Serious adverse reactions including cardiovascular and neurologic toxicity have occurred infrequently, primarily at higher doses. Hematologic toxicity and elevations in liver function parameters were also observed, but rarely required dose attenuation. Adverse effects were usually reversible after dose reduction or discontinuation of therapy. A summary of clinical trial results will be presented.

Gene Regulation II

M6 NEWLY REPLICATED DNA EXHIBITS INCREASED SENSITIVITY TO NUCLEASE DIGESTION IN DAUDI CELLS DURING GROWTH INHIBITION BY INTERFERONS, Michael J. Clemens and Ruth E. Exley, Cancer Research Campaign Group, St.George's Hospital Medical School, London SW17 ORE, U.K.

In interferon-treated Daudi cells, DNA replication becomes uncoupled from cell division and there is a rapid turnover of up to 50% of the newly synthesized DNA. This could be due either to increased cellular nuclease activity or to a greater susceptibility of the newly replicated DNA to existing nucleases. When control cells are pulse-labelled with thymidine the newly synthecised chromatin undergoes a series of structural changes which alter its sensitivity to micrococcal nuclease digestion in isolated nuclei. Initially, newly labelled DNA is protected from digestion, perhaps because of association of replication forks with the nuclear matrix. Within 5-10 min this DNA transiently becomes more nuclease-sensitive, as the chromatin undergoes structural maturation. Within 20 min, the DNA acquires the nuclease-resistance characteristics of bulk parental chromatin. In contrast, in interferon-treated Daudi cells from 2 to 30 min of labelling the DNA in isolated nuclei shows increased susceptibility to nuclease digestion, suggesting that it is less protected by its association with histones or other proteins found in mature chromatin. No difference is observed in nuclease sensitivity of bulk chromatin between control and interferon-treated cells, and normal nucleosome ladders are observed on agarose gel electrophoresis of the digestion products. These results suggest that DNA turnover in interferon-treated Daudi cells is a consequence of defective assembly of newly replicated DNA into mature chromatin, perhaps because of an impairment in chromatin protein synthesis.

M7 A NOVEL, GROWTH RELATED GLYCOPROTEIN WHOSE SYNTHESIS IS INHIBITED BY INTERFERON, Ara G. Hovanessian, Eliane Meurs, Josette Svab and Anne G. Laurent, Unité d'Oncologie Virale, Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France

Using a monoclonal antibody we could describe here a novel, 84,000-Mr glycoprotein (gp84) localized on the surface of human Daudi cells. The identity of this gp84 is not known. Its synthesis is stimulated following the addition of serum to serum-starved cells. The synthesis of gp84 is inhibited 4-6 hours after the addition of interferon to Daudi cells sensitive to the anti-growth effect of interferon. Under these experimental conditions the overall protein synthesis is normal and the double-stranded (ds)RNA-dependent protein kinase and the 2-5A synthetase are induced. Interferon treatment of Daudi cells does not affect the turnover nor the glycosylation of gp84. *In vitro* translation of total polyadenylated RNA from control and interferon-treated cells indicated that this inhibition is due to a reduced amount of gp84 mRNA.
The specific inhibition of gp84 is correlated with the anti-growth action of interferon. The synthesis of gp84 is not affected in interferon-treated Daudi cells resistant to the anti-growth action of interferon. In these resistant cells however, both the dsRNA-dependent protein kinase and the 2-5A synthetase are induced by interferon.

M8 ACTIVATION AND INHIBITION OF GENE EXPRESSION BY INTERFERONS, P. Lengyel, S. Tominaga*, H. Samanta**, D. Engel, H. Chao, M. Garcia-Blanco, and A. Thakur, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511

We have isolated the 5' terminal segment with flanking sequences of a mouse gene (gene 202) which specifies mRNAs (202 mRNAs) whose level is over 30 fold higher in Ehrlich ascites tumor cells exposed to IFN than in control cells (1,2). The attachment of part of this segment from the 202 gene rendered the expression of the chloramphenicol acetyltransferase gene responsive to IFN. Studies with primer extension and S1 protection revealed multiple 5' termini in 202 mRNAs. Most of these map to an about 170 nucleotide long region. This region and its vicinity are free from TATA boxes. Treatment with INF increased the level of the 202 mRNAs with different 5' termini nonuniformly.

Treatment with IFN changed the pattern of secreted proteins from quiescent BALB/c-3T3 cells and from BALB/c-3T3 cells treated with a partially purified platelet derived growth factor preparation (PDGF): it inhibited the accumulation of the PDGF induced proteins (including a 63 kDa protein) and it induced the accumulation of several other proteins (including proteins of 30 and 89 kDa) (3). The 30 and the 89 kDa proteins were partially purified. The 89 kDa protein shared at least one characteristic with some INF-induced cell-associated enzymes: it bound double stranded RNA tightly. The 63 kDa protein was obtained in a radioactively pure form. The protein was undetectable in the culture fluid from resting BALB/c-3T3 cells and was barely or not at all detectable in the culture fluid from growing BALB/c-3T3 and NIH 3T3 cells respectively. The protein was, however, among the three major, constitutively secreted proteins in the case of growing, Kirsten murine sarcoma virus transformed NIH 3T3 cells. Treatment with 1,000 units per ml of beta IFN decreased the accumulation of the 63 kDa protein in the culture fluid of quiescent BALB/c-3T3 cells which had been treated with PDGF by over 80% and that in the culture fluid of Kirsten murine sarcoma virus transformed NIH 3T3 cells by about 50%. This decrease was not a consequence of an inhibition of cell growth (4).

(1) Samanta, H., J.P. Dougherty, M.E. Brawner, H. Schmidt, and P. Lengyel UCLA Symp. on Molecular and Cellular Biology, Vol XXV, 59-72 (1982) (2) Engel, D., H. Samanta, M. Brawner and P. Lengyel, Virology 142, 389-397 (1985). (3) Tominaga, S., and P. Lengyel, J. Biol. - Chem. 260, 1975-1978 (1985). (4) Tominaga, S., K. Tominaga, and P. Lengyel, J. Biol. Chem. (in press). *JICHI Medical School, Tochigi, Japan;**Eugene Tech International, Allendale, New Jersey.

M9 INTERFERON-ACTIVATED (2'-5') OLIGO A SYNTHETASE GENE AND ITS VARIATION DURING CELL GROWTH, Michel Revel, Judith Chebath, Philippe Benech, Valerie Wells*, Livio Malluci, Department of Virology, The Weizmann Institute of Science, Rehovot, Israel

We previously reported the spontaneous secretion of IFN-β by cells undergoing growth-transition during the differentiation of hemopoietic cells, and proposed that this IFN works as an autocrine inhibitor of cell growth (1).Kimchi et al. (this symposium) have confirmed that blocking of the autocrine IFN by antibodies prevents the arrest of cell growth and the switch-off of the c-myc protooncogene. Rise in (2'-5') oligo A synthetase (OASE) and HLA class I have been used as markers of the autocrine IFN action in differentiating U937 histiocytic cells (1). Endogenous IFN and OASE increase are also seen during the normal cell cycle of mouse embryo fibroblasts (MEF) synchronized tertiary cultures where OASE rises sharply late in S phase and falls again in G2. We have used cloned OASE cDNA probes to study the regulation of the gene by endogenous and exogenous IFNs. Analysis of the human OASE gene has revealed that two IFN-induced active mRNAs of 1.6 Kb (E16) and 1.8 Kb (E18) are produced by a differential splicing mechanism which shows cell specificity (2,3). The E16 RNA is seen induced by the autocrine IFN in differentiating U937 cells (1). E16 cDNA codes for a 40 Kd (363 aa) protein with a hydrophobic C-terminus; expressed in E. coli, it produces ds RNA-binding OASE activity demonstrating that IFN indeed induces the structural gene for the enzyme. E18 cDNA codes for a 46 Kd identical to E16 in its first 346 aa but terminating by an acidic domain which could interact with basic proteins in the cell. Antibodies against synthetic peptides, derived from the E16 and E18 sequences, demonstrate on Western blots the existence of the two 40 and 46 Kd proteins induced by IFN and binding to ds RNA, but also reveals larger polypeptides, which could correspond to the larger forms of the OASE observed in human and mouse cells. E16 and E18 cDNAs hybridize to several large RNAs (2.7, 3.6 and 4 Kb) induced by IFN in human cells, but some of these may be incompletely spliced precursors (2). In mouse cells, OASE cDNAs detect a 1.7 Kb RNA and two large RNAs of 4 and 5 Kb. While the 1.7 Kb is preferentially induced by exogenous mouse IFN, the RNAs which correlate with the OASE peak in the S phase of synchronized MEF are exclusively the 4 and 5 Kb species. Thus, large OASE RNAs are active in mouse cells, and may code for a form of the enzyme specifically involved in some cell-cycle related function, as histone mRNA degradation in preparation for mitosis. Different forms of OASE may be induced during cell-cycle and in the growth-arrest during differentiation.

(1) Yarden, A., Shure-Gottlieb, H., Chebath, J., Revel, M., and Kimchi, A. (1984) EMBO J. 3, 969-973.
(2) Benech, P., Merlin, G., Revel, M., and Chebath, J. (1985) Nucl.Acids Res. 13, 1267-1281.
(3) Benech, P., Mory, Y., Revel, M., and Chebath. J. (1985) EMBO J. 4, 2249-2256.

*Guys Hospital Medical School, London Bridge, England.

M10 INTERFERON MEDIATED REGULATION OF GENE EXPRESSION IN MOUSE CELLS, T. Sreevalsan, A. Masibay, L. Tumarkin and G.P. Damewood, IV, Department of Microbiology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007

At present the basis of interferon's (IFN) antiproliferative effects remains poorly understood. The pleiotropic effects of IFN on gene expression should play a role in its antiproliferative effects. Kinetic analysis of mRNA: cDNA hybridization indicates that about 3% of the mRNAs found in growing mouse cells are absent in resting cells (1): a finding consistent with the recent identification of several cell-division cycle (cdc) genes in animals cells (2,3,4). We have begun to characterize those cdc genes sensitive to IFN's action in Swiss mouse 3T3 cells. We constructed a complementary DNA (cDNA) library using mRNAs appearing in resting 3T3 cells stimulated to proliferate. The cDNA library was probed with three distinct ^{32}P labeled single stranded cDNAs synthesized using poly A^+ RNA from a) resting cells, b) resting cells stimulated with serum to proliferate and c) resting cells stimulated with serum and IFN to proliferate. Additionally, similar ^{32}P labeled cDNA probes were used to screen a mouse 3T3 cell genome library constructed using EMBL4 Lambda vector.
Eleven independent recombinants from the cDNA library were expressed in serum-stimulated cells only and expression of eight of them was inhibited in IFN-treated cells. Based on the insert size and the restriction pattern these clones are different from each other. The size of the mature transcripts in 3T3 cells corresponding to the various cDNA clones varied from $1.0x10^5$ daltons to $1.8x10^6$ daltons.
We detected ten IFN-sensitive genes by screening the mouse genomic library. These genes were expressed preferentially in resting cells stimulated with serum. Based on the insert size and restriction patterns these genes are different from each other.
The extent and kinetics of synthesis of the individual mRNAs in IFN-sensitive and IFN resistant 3T3 cells are being examined at present to determine their significance in IFN's antiproliferative effects.

1) Williams, J.G. and Penman, S. (1975) Cell, 6, 197.
2) Cochran, B.H., Reffel, A.C. and Stiles, C.D. (1983) Cell, 33, 939.
3) Linzer, D.I.H. and Nathans, D. (1983) Proc. Natl. Acad. Sci. (U.S.A.) 80, 4271.
4) Hirschhorn, R.R., Patricio, A., Yuan, Z., Gibson, C.W. and Baserga, R. (1984) Proc. Natl. Acad. Sci. (U.S.A.) 81, 6004.

Structure-Activity Relationships of Interferons and Interferon Receptors

M11 CHARACTERIZATION OF MURINE INTERFERON GAMMA RECEPTORS.
Michel Aguet (1) and Juana Wietzerbin (2). 1 Institute of Immunology and Virology, University of Zurich, Switzerland, 2 Institut Curie, Paris, France.
Pure recombinant E. coli derived mouse IFN gamma (Genentech, Boehringer Ingelheim) was labelled with ^{125}I to a specific radioactivity of about 50 uCi/ug without detectable loss of its antiviral activity. Binding studies on exponentially growing mouse leukemia L1210 cells revealed specific non-cooperative binding to approximately 10^4 binding sites per cell with an average binding constant of about 10^{-10} M at 4°. Mouse L929 cells, normal mouse fibroblasts, normal mouse spleen cells and unstimulated peritoneal or bone marrow macrophages displayed similar binding properties. Normal cells lacking homologous high affinity IFN gamma receptors could not be detected as yet. Upon treatment at low pH receptor bound IFN was displaced from L1210 cells incubated with labelled IFN gamma at 4°. Incubation at 37°, however, rendered specifically bound IFN refractory to dissociative conditions, compatible with ligand internalization. Receptor expression was quantitatively dependent on the growth phase with maximum expression on exponentially growing L1210 cells. Pretreatment of L1210 cells with nonsaturating doses of unlabelled mouse IFN gamma resulted in a pronounced receptor down-regulation corresponding to a 90 % reduction of the binding capacity. Cross-linking of ^{125}I labelled mouse IFN gamma to its mouse L1210 cell receptors yielded a labelled complex of about M_r 110,000 upon SDS-PAGE. The formation of this complex was prevented by addition of excess unlabelled IFN gamma but not IFN alpha/beta which binds to distinct receptors. Moreover, this complex could not be extracted from L1210 cells with down-regulated IFN gamma receptors.

M12 INTERFERON-GAMMA BINDS TO HIGH AND LOW AFFINITY RECEPTOR COMPONENTS ON MOUSE MACROPHAGES. Ramani A. Aiyer and Patricia P. Jones, Dept. of Biol. Sciences, Stanford University, Stanford, CA 94305.

Interferon-γ, a glycoprotein secreted by activated T lymphocytes, activates many macrophage functions, and modulates immune responses. In the mouse macrophage cell line WEHI-3 murine interferon-γ (MuIFN-γ) induces the increased expression of Ia and H-2 antigens of the major histocompatibility complex (MHC). Using [^{125}I]-MuIFN-γ, we have identified specific receptors on WEHI-3 and other mouse cells. Binding of [^{125}I]-MuIFN-γ was: 1) time- and temperature-dependent, 2) species-specific, and 3) competitively inhibited by "cold" MuIFN-γ, but not MuIFN-α or MuIFN-β. Analysis of steady-state binding, at 37°C, yielded a curvilinear Scatchard plot, consistent with the presence of two classes of binding sites with K_ds of 9.1 x 10^{-11} M (500 sites/cell) and 2.7 x 10^{-9} M (4400 sites/cell). This result differs significantly from that obtained by various other groups that have reported only a single class of IFN-γ binding sites with K_ds ranging from 10^{-8} M to 10^{-10} M. Comparison of MuIFN-γ binding with the dose-response for MHC antigen induction on WEHI-3 cells indicates that the higher affinity sites most likely represent the physiologically relevant MuIFN-γ receptors. Furthermore, the half-maximal biological response occured at a fractional occupancy by MuIFN-γ of only 5% of the high-affinity receptors.

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M13 RECEPTORS FOR HUMAN INTERFERONS, Sohan L. Gupta, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Purified recombinant human interferons (HuIFNs) have been used to study IFN receptors on human cells. Binding and competition experiments with ^{125}I-labeled IFNs have indicated that IFNs bind to specific receptors on cells with high affinity. Cross-linking of ^{125}I-labeled HuIFNs bound to human cells (Daudi, WISH, etc.) yielded specific complexes of Mr ~ 140,000-150,000 with HuIFN-α_2 or HuIFN-β Ser_{17} and Mr ~ 110,000 with HuIFN-γ. Competition experiments have provided evidence indicating that HuIFN-α and -β bind to common receptors whereas HuIFN-γ interacts with different receptors, and that the species specificity of mouse and human IFNs may reside at the receptor level. Mouse-human hybrid cells containing three copies of human chromosome 21 as the only human chromosomes expressed receptors for HuIFN-α/β, and developed antiviral state upon treatment with HuIFN-α or -β; however, these hybrid cells did not display receptors for HuIFN-γ and were completely insensitive to the antiviral action of HuIFN-γ, suggesting that the receptors for HuIFN-α and -β but not for HuIFN-γ are specified by human chromosome 21.

The following results suggest that the 140,000-150,000 Mr complex formed with ^{125}I-labeled HuIFN-α_2 may form non-covalent dimers in the plasma membrane: (i) the ^{125}I-HuIFN-α_2-receptor complex solubilized with Triton X-100 eluted from Sephacryl S-500 column at about the same position as thyroglobulin (Mr ~ 669,000); (ii) cross-linking of cell bound ^{125}I-HuIFN-α_2 at increasing concentrations of the cross-linker (disuccinimidyl suberate) yielded a second complex of Mr ~ 300,000 in addition to the 150,000 Mr complex. The formation of both 150,000 and 300,000 Mr complexes appears to be specific; (iii) the 300,000 Mr complex was obtained with HuIFN-α_2 as well as HuIFN-β Ser-17, in agreement with the view that HuIFN-α and -β bind to the same receptors; (iv) evidence was obtained indicating that the 300,000 Mr complex was formed from 150,000 Mr complex. It is conceivable that the formation of the 300,000 Mr complex represents a step in the sequence of events initiated upon IFN binding to receptors.

HuIFN-α_2-receptor complex formed on incubation with cells at 4°C was rapidly internalized upon shifting to 37°C. This was accompanied by a loss of HuIFN-α_2 receptors on the cell surface. This down regulation of HuIFN-α_2 receptors was brought about by HuIFN-α or HuIFN-β but not by HuIFN-γ. The recovery of receptors on the cell surface, following down regulation, took several hours and required protein synthesis suggesting that HuIFN-α/β receptors do not recycle and new receptors had to be synthesized. A loss of receptors from the cell surface upon IFN treatment could, at least in part, account for the time-dependent decline in IFN-mediated induction of cellular gene expression and could provide a self-regulating mechanism for the cellular response to IFN.

M14 MULTIPLE HUMAN INTERFERON-γ RECEPTORS. Menachem Rubinstein, Dina G. Fischer and Patricia Orchansky. Dept. of Virology, Weizmann Institute of Science, Rehovot, Israel
The receptor for interferon-γ (IFN-γ) was characterized in a variety of human cells and cell lines with the aid of ^{125}I-IFN-γ. Two types of receptors were identified and distinguished by their ability (or lack of it) to bind denatured IFN-γ. Human fibroblasts, low density lymphocytes and the amnion cell line WISH bound both natural and denatured IFN-γ with a similar affinity. Scatchard analysis gave a linear plot, indicating a single class of high affinity ($Kd \leq 10^{-10}$M) binding sites. Denatured IFN-γ (pH2, 4°C, 24 hr) lost its antiviral activity but along with the binding it retained the ability to induce HLA-DR in these cells. In contrast, human monocytes and the B-lymphoblastoid cell line Daudi bound only natural IFN-γ and Scatchard analysis gave a non linear plot, indicating multiple binding sites. Moreover, IFN-γ neither induced (2'-5') oligo A synthetase nor an antiviral state in monocytes and in Daudi cells. It was therefore concluded that there are at least two different receptors for IFN-γ in human cells both in terms of specificity and function. The fate of bound IFN-γ was studied in WISH cells. Internalization at 37°C was very slow but a rapid degradation of IFN-γ was observed. This degradation was not endosomal since it was not inhibited by either chloroquin or methylamine. Finally, the receptor binding epitope on IFN-γ was localized by a variety of proteolytic procedures. It was found that the first two N-terminal amino acids were essential for both binding and activity while the C-terminal amino acids (positions 125-143) were less critical for either activity or binding. The data can be used to design specific antagonists of IFN-γ which act by reversibly blocking the binding of IFN-γ to its receptors.

M15 STRUCTURE-FUNCTION RELATIONS IN RECOMBINANT GAMMA INTERFERONS, Paul P. Trotta, Gail F. Seelig, Hung V. Le and T.L. Nagabhushan, Schering-Plough Corp., Bloomfield, N.J. 07003
Structure-function relations have been studied by combining epitope analysis with a monoclonal antibody (Mab) to recombinant human gamma interferon (HuIFNγ) and biological activity measurements of cloned truncated variants of HuIFNγ and murine gamma interferon (MuIFNγ). The Mab described here binds to the full-length (146 amino acid residues) HuIFNγ (HuIFNγ-A) as well as to a 15-residue C-terminal polypeptide fragment but does not recognize a variant terminating at position 131 (HuIFNγ-B). The Mab binds to a cyanogen bromide fragment of this polypeptide corresponding to residues 132-137 in HuIFNγ-A (Arg-Lys-Arg-Ser-Gln-Met) as well as to a truncated variant lacking the 5 C-terminal amino acids. It does not recognize a polypeptide corresponding to the 9 N-terminal amino acids. The Mab neutralizes antiviral activity measured in a cytopathic effect-inhibition assay. It is consistent with these data that the specific antiviral activity of HuIFNγ-B is significantly lower than the corresponding value for HuIFNγ-A. In parallel experiments we have purified to homogeneity four MuIFNγ variants: the full-length 136 amino acid residue protein (MuIFNγ-A), a form lacking the first three amino acids (Cys-Tyr-Cys) at the N-terminus (MuIFNγ-D) and two products truncated at the C-terminus (MuIFNγ-D' and -D"). Primary sequence analysis by Edman degradation with a gas phase sequenator has indicated that MuIFNγ-D' and -D" terminate at residues 130 and 125, respectively (residue numbers correspond to positions in MuIFNγ-A) (1). The specific antiviral activities of purified MuIFNγ-A, -D, -D' and D" are 2, 20, 20 and 4 x 10^6 units/mg, respectively. These data indicate that selected residues may be cleaved from N- and C-termini in MuIFNγ-A without loss in antiviral activity, but that at least a portion of residues 126-130 (Ser-Ser-Leu-Arg-Lys) are required for maximal activity. Thus, we have elucidated various aspects of the structure-function relations in both HuIFNγ and MuIFNγ.

(1) Bond, M.W. (1985) Symposium of American Protein Chemists, Abst. 109

M16 INTERFERON RECEPTOR REGULATION OF GENE TRANSCRIPTION, Bryan R.G. Williams and Greg Hannigan, Division of Infectious Diseases, Research Institute, Hospital for Sick Children and Department of Medical Genetics, University of Toronto, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

Interferons (IFNs) exert potent growth inhibitory and antiviral activities on cells with which they interact. This interaction of IFN and cell is mediated through specific high-affinity receptors in the plasma membrane. As a first step to understanding the role of the IFN receptor in mediating the cellular responses to IFN, we have analyzed in detail the relationship between IFN-α receptor expression and the transcriptional response to this IFN of the 1-8 gene family (1) and the gene for 2-5A synthetase (2). We have utilized the human glioblastoma cell line, T98G (from which the 1-8 sequences were originally isolated), as it is sensitive to both the antiviral and growth inhibitory effects of IFN-α. Moreover, this line is interesting because, although transformed and capable of growth in soft agar, it displays the characteristic of density-dependent growth arrest (3). By allowing the cells to reach the arrested state we have noted significant alterations in the expression of IFN-α receptors, relative to that observed on growing cultures. Cultures moving from exponential growth into a state of density-dependent growth arrest, demonstrate a 7-fold drop in the total number of IFN-α receptors expressed per cell. This loss of receptor activity was not seen when cells were grown in the presence of an anti-IFN-α monoclonal antibody. The active binding sites expressed on the arrested cell population were of reduced affinity for IFN, relative to the high-affinity sites expressed on the growing cells, resulting in a 3-fold lower initial rate of IFN-binding to the arrested cells. Thus, there is regulation of IFN-α receptor expression by endogenously produced IFN in a negative autocrine manner in T98G cells. We exploited the difference in receptor expression to investigate the relationship between IFN-receptor binding and induced gene transcription. As determined by nuclear runoff assay, the transcriptional response of both the gene family 1-8, and 2-5A synthetase, to IFN treatment also showed a 3-fold reduction in density arrested cells. Longer-term (0-8hr) induction and down-regulation of gene transcription in IFN-treated cells closely followed the binding to, and down-regulation of, cell-surface localized IFN receptors. Thus, transcription of these IFN-induced genes is closely linked to receptor occupancy, and is most likely mediated by transmembrane signals alone.

1. Friedman, R.L. et al (1984). Cell 38, 745-755.
2. Saunders, M.E. et al (1985). EMBO J. 4, 1761-1768.
3. Stein, G.H. (1979). J. Cell. Physiol. 99, 43-54.

Biological Activities of Interferon and Animal Models — I

M17 MECHANISMS OF ANTITUMOUR ACTION OF INTERFERONS OF HUMAN TUMOUR XENOGRAFTS AND IN MOUSE METASTASES MODELS. F.R. Balkwill[1], M. Stevens[1], E. Proeitti[2], J. Bodmer[1], I. Hart[1], P. Ramani[1]. [1]Imperial Cancer Research Fund, London, WC2A 3PX. [2]Instituto Superiore di Sanita, 00161, Rome.

Interferons, IFNs, may inhibit tumour growth by directly affecting the behaviour of malignant cells or by modulating the host response to such cells. Human alpha IFNs will inhibit the growth of human tumours growing subcutaneously in nude mice and this appears to be due to direct growth inhibition. In the majority (7/10) of early passage xenografts obtained from primary human tumours IFN-αs were tumour static but caused complete regression in two tumour lines. The IFN-αs had no measurable effect on the murine host, but strongly increased levels of IFN induced enzyme in the human tumour. Recombinant IFN-γ did not generally inhibit growth of the xenografted tumours (1/10 responded to this IFN) but other direct effects on the tumour were measurable. For instance systemmic IFN-γ induced or enhanced expresson of HLA-DR antigens on a breast and a bowel cancer xenograft growing subcutaneously and tumour associated antigens on another bowel cancer xenograft. Three different preparations of murine IFN also inhibited human tumour cell growth in nude and beige nude (NK deficient) mice and this inhibition was not related to sensitivity of tumours to human IFN-αs. There was no evidence that murine IFN had a direct effect on the tumours e.g. murine IFN did not enhance HLA expression on the human xenograft cells in vitro. Further investigations into this (presumably) host mediated effect are currently underway in mice with varying degrees of immunosupression and deficiency. We have also investigated the antimetastatic effect of the recombinant human IFN-α A/D hybrid which is reactive on murine cells, and murine rIFN-γ, in a metastasis model. These two IFNs strongly inhibited the development of experimental pulmonary metastases of the COLO 26 adenocarcinoma cell line when administered x 5 weekly after tumour cell injection in Balb-c, Balb-c nude and beige (NK deficient) nude mice. Studies with mice subject to further immune depletion are currently underway. In vitro COLO 26 cells were resistant to NK cell mediated lysis but sensitive to cytotoxic macrophages and the cytostatic effects of rIFN-α A/D. Kinetic studies showed that administration of rIFN-α A/D during the first 5 days post injection was of crucial importance, and studies with labelled COLO 26 showed that IFN treated mice cleared tumour cells more effectively from the lungs than control mice. This tumour spontaneously metastasizes to the lungs from a subcutaneous site and preliminary studies indicate that IFN can inhibit this process as well. Although the mechanism of the antimetastic effect of IFNs is still not elucidated, we have evidence that human IFNs α and γ can prevent experimental metastasis of a human melanoma cell line injected intravenously in nude mice, but have no measurable effect on the murine host. It is hoped that by studying these complementary animal models some insight into anticancer effects of IFNs may be attained.

M18 IN VIVO TREATMENT OF MURINE LYMPHOMA WITH INTERFERONS AND WITH MONOCLONAL ANTIBODIES, Teresa Basham, Mark Kaminski, Ronald Levy and Thomas Merigan, Division of Infectious Diseases and Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305

Interferon and monoclonal antibody therapy have shown significant antitumor responses in nodular lymphoma patients, although neither treatment modality has been completely successful. As interferon has immunomodulating as well as antiproliferative activity, it was possible that combination therapy of interferon and monoclonal anti-idiotype antibody would cooperate synergistically in vivo and they should at least be additive. We have studied these two immunomodulatory agents, alone and in combination, in an in vivo murine B cell lymphoma model, 38C13. Our results indicate that anti-idiotype monoclonal antibodies of the IgG2a isotypes are superior to antibodies of the IgG1 and IgG2b isotypes both in protecting animals against the tumor and in eliciting macrophage antibody dependent cellular cytotoxity (ADCC). Recombinant interferon α A/D Bgl, which has direct antiproliferative activity for 38C13 in vitro, gave some in vivo protection at doses of 10^5 - 10^6 units/mouse/day on a 3 times weekly schedule. In vivo administration of rIFN-α A/D Bgl also enhanced macrophage ADCC. Combination therapy of IgG2a anti-idiotype and r-IFN-α A/D Bgl appears to be synergistic when survival is compared to that of the individual treatments. In addition, interferon made active an IgG2b anti-idiotype monoclonal antibody which alone had no protective activity against the tumor invivo.

M19 EXPRESSION OF INTERFERON STRUCTURAL AND REGULATORY GENES IN THE MOUSE, Edward De Maeyer, Françoise Dandoy and Jaqueline De Maeyer-Guignard, Institut Curie, Biologie, Campus d'Orsay, Bât. 110, 91405 Orsay (France).

The murine IFN-α structural gene cluster (Ifa) has recently been located on chromosome 4. Segregation of RFLP in genomic DNA from recombinant inbred and congenic lines has revealed linkage of Ifa to H-15, near "brown". We have now finished the fine mapping of Ifa by following segregation of Ifa RFLP and 3 other loci (Mup-1, brown and misty) in two different backcrosses. The gene order is : Mup-1, brown, Ifa and misty. It was not possible to do the fine mapping of the Ifb locus using the same backcross progeny that was used for the mapping of the Ifa locus, because of lack of RFLP between BALB/c, DBA/2 and C57BL/6 genomic DNA when probed with the MuIFN-β cDNA. However, genomic DNA from a species of wild mice (mus Spretus Lataste) restricted with HindIII and probed with the murine IFN-beta probe, did display RFLP when compared to laboratory mice. Although mus Spretus belongs to a different species, certain interspecies crosses with mus musculus are possible, and we were therefore able to follow segregation of RFLP in HindIII restricted genomic DNA from backcross progeny (DBA/2 x M. Spretus) F1 x DBA/2 probed with a Murine IFN-alpha and IFN-beta cDNA. There was complete coincidence between the segregation of parental and F1 type IFN-α and IFN-β RFLP, indicating tight linkage of Ifa and Ifb.

The localization of the MuIFN-α and IFN-β genes has enabled us to investigate the relative contribution of IFN structural genes of If-loci to the quantitative regulation of circulating IFN production. Previous studies have shown that serum IFN production in mice is quantitatively influenced by If loci, whose alleles determine high or low production. Although different loci influence IFN production in response to different inducers such as NDV, Sendai virus, HSV-1 and polyrIrC, BALB/c mice are in every instance low producers. It was therefore possible that, in addition to If-loci, some feature of the BALB/c structural IFN genes contributed to low production. This was examined by measuring IFN production in two strains of C57BL/6 mice, congenic with BALB/c at the MuIFN-α gene cluster on chromosome 4. One line, HW 13 (B6.C-H-15c-H-16c-H-20c-H-21c/By) has a BALB/c fragment on chromosome 4 of at least 35 cM which includes the BALB/c IFN-α gene cluster ; the other line, HW 13 J (B6.C-H-15c/By), has a much shorter fragment of about 15 cM, also comprising the BALB/c IFN-α gene cluster. These mice, carrying the BALB/c IFN-α structural genes on a C57BL/6 background, are high IFN producers when stimulated by NDV, Sendai virus, HSV-1 or polyrIrC. Thus, the low IFN production of BALB/c mice is not directly due to some feature of the IFN structural genes, but is mainly the result of different alleles at If loci, not linked to the structural genes.

Using in situ hybridization techniques with ^{35}S labelled IFN-α and IFN-β cDNA probes, we have examined the cellular basis for high and low IFN production in $If\text{-}1^h$ and $If\text{-}1^l$ congenic mice, with the aim of obtaining information on the number of IFN producing cell in high and low responders.

Biological Activities of Interferon and Animal Models — II

M20 LEUKOCYTE MEDIATION OF INTERFERON'S ANTITUMOR ACTIONS, Samuel Baron, T.K. Hughes, M. Sarzotti-Kelsoe, G.R. Klimpel, W.R. Fleischmann, Jr., D. Weigent, G.J. Stanton, and S. Tyring, Department of Microbiology, University of Texas Medical Branch, Galveston, TX 77550

Interferon (IFN) may exert antitumor actions by a number of mechanisms including: a) inhibition of tumor virus multiplication; b) decrease of division rate of tumor cells; c) direct cytolysis of many tumor cells mainly by IFN γ; d) indirect cytolysis of tumor cell by activation of several effector cells (NK, K, ADCC, sensitized T lymphocytes, macrophages, and another group of peripheral blood leukocytes (PBL)); e) hormonal effects; f) immunomodulatory effects; and g) modulation of cell membrane macromolecules. We are studying the indirect cytolysis of cultured tumor cells treated with IFNs and leukocytes in assays that measure target cell killing by enumeration of viable cells and radioisotope release. Our studies are centered on the IFNγ activation of PBL because of its unusual characteristics. This cytolysis is induced mainly by IFNγ and uncommonly by IFNs α or β. The dose of IFNγ required to activate PBL range from a minimum of 5 U/ml to a maximum of 200 U/ml. The majority of primary, secondary, and continuous human tumor cells are lysed by this mechanism but normal cells are lysed to a lesser degree. IFNγ itself is the probable inducing mechanism, because the cytolysis can be induced with purified recombinant IFNγ and because antibody specific to the synthetic IFN peptide inhibited the effect. Cytolysis is not detectable before 16 hours, thereby distinguishing this cytolytic mechanism from the 4 hour killing by NK, K, LAK, and sensitized T lymphocytes. Depletion of nylon wool adherent PBL (macrophages and B cells) did not eliminate cytolysis by the IFNγ activated PBL. The relative degree of antitumor action in vitro is observed to be similar to that in vivo using two mouse tumors. Perhaps most interesting is the finding that unlike most other actions of IFN, effector leukocytes could not be fully activated by preincubation with IFN γ. The effector cell may be related to the T effector cell previously reported (1). These findings suggest the existence of a distinctive cytolytic action of effector lymphocytes that is characterized by: a) induction mainly by IFN γ, b) a minimum of 16 hours for cytolysis to begin, c) inability of IFNγ to fully preactivate effector PBL, d) activity against most human tumors in culture, and e) correlation of the antitumor action in vitro and in vivo using two mouse tumors.

1. Weigent, D.A., M.P. Langford, W.R. Fleischmann and G.J. Stanton. 1982. Enhancement of natural killing activity by different types of interferon. In Human Lymphokines by N.O. Hill, A. Kohn, and D.C. Dumonde (eds.). Academic Press, N.Y. p. 539-550.

M21 INTERFERON-INDUCED INHIBITION OF CHLAMYDIA TRACHOMATIS: DISSOCIATION FROM OTHER BIOLOGICAL ACTIVITIES OF INTERFERONS, Christine W. Czarniecki, Ellena M. Peterson and Luis M. de la Maza, Department of Pharmacological Sciences, Genentech, Inc., South San Francisco, CA 94080 and Department of Pathology University of California, Irvine, CA 92717.

The three classes of interferons (IFNs), α, β, γ, have been shown to inhibit the proliferation of cells as well as the replication of viruses in cells treated with these proteins. Our studies with purified E. coli-derived IFNs α, β, γ, confirm earlier reports by others that indicated that IFNs also inhibit the growth of intracellular pathogens more complex than viruses. Treating murine (McCoy) cells with murine IFN-γ resulted in a dramatic inhibition of growth of Chlamydia trachomatis. Greater than 95 percent inhibition was obtained with only 1 unit/ml of IFN. This dose inhibited cell growth by only 15 percent and concentrations up to 10^4 units/ml had minimal effect on the replication of encephalomyocarditis virus. The dissociation of IFN-induced biological activities was also demonstrated in human (HeLa) cells treated with various human IFN-α subtypes. IFN-α1 had reduced antiviral and antiproliferative activities on HeLa cells, in comparison to IFN-α2. However, only IFN-α1 inhibited the growth of Chlamydia trachomatis. The hybrid molecule IFN-α2/α1 (Bgl II) had better antiviral and antiproliferative activities than either parental IFN but had no detectable antichlamydial activity in HeLa cells. In contrast, the hybrid IFN-α1/α2 (Bgl II) had reduced antiviral and antiproliferative activities but its antichlamydial activity was as potent as that of IFN-α1 in these cells. In view of these results it is tempting to speculate that it

may, one day, be possible to design novel IFN molecules which have lost specific activities and retained others.

Ultrastructural analysis of the growth cycle of Chlamydia trachomatis in IFN-treated and untreated cells indicated that the IFN-induced inhibition occurred primarily at the stage of transformation from elementary bodies to reticulate bodies, resulting in a marked decrease in the number of chlamydial inclusions. The IFN-treated cells contained fewer and more immature chlamydial forms than the control cells and secondary infection of previously uninfected cells could not be detected in IFN-treated cells.

M22 MECHANISM OF ANTIPROLIFERATIVE ACTION OF INTERFERONS, Mariano Esteban and Eduardo Paez, Departments of Biochemistry, Microbiology and Immunology, Downstate Medical Center, Brooklyn, New York 11203.

The molecular mechanisms responsible for the antiproliferative action of interferons (IFN) are unknown. We have used vaccinia virus as model to study the effects of IFN on DNA related processes. This is because vaccinia is a large (180 Kb) DNA-containing virus that replicates in the cell cytoplasm, the DNA codes for many of the enzymes required for viral RNA and DNA biogenesis, and the virus growth is resistant to IFN treatment in various cultured cells. We showed that in spite of a lack of effect of IFN on vaccinia virus RNA and protein synthesis, IFN still causes a decrease in elongation of replicating viral DNA (1). IFN also blocked marker rescue of ts mutants of vaccinia virus, suggesting an effect of IFN on homologous DNA recombination (2). Moreover, while large deletions at the left terminus of vaccinia DNA occurred readily during vaccinia virus persistence in Friend erythroleukemia (FEL) cells (3), IFN completely suppressed the generation of these spontaneous deletions (4). In addition, we have used gene-transfer to examine whether IFN inhibits the transforming potentiality of activated human oncogenes, the level of this inhibition and whether IFN discriminates among viral genes, cellular genes and oncogenes. In gene transfer experiments IFN severely inhibited to similar extent the stable transformation of Ltk(-) and NIH3T3 mouse cells by the chicken tk gene, Ecogpt gene, SV-40, v-Ha-ras and human c-Ha-ras and c-ki-ras oncogenes (5). This inhibition occurs at the level of stabilization, integration or both of exogenous DNA sequences in the recipient cells, with an apparent effect on gene expression. By decreasing DNA elongation rates, preventing DNA deletion formation and blocking ligation-integration of DNA, IFN provides a mechanism of action which might explain some of its own antiproliferative properties. Experiments are underway to determine whether IFN alters the activity of enzymes involved in DNA replication.

1. Esteban, M. (1984) J. Interferon Research 4, 179-192.
2. Paez, E. and Esteban, M. (1985). J. Interferon Research 5, 247-256.
3. Paez, E., Dallo, S. and Esteban (1985). Proc. Natl. Acad. Sei. USA 82, 3365-3369.
4. Paez. E. and Esteban, M. (1985). J. Virol. 56, 75-84.
5. Perucho, M. and Esteban, M. (1985). J. Virol. 54, 229-232.

M23 Recombinant Human Interferon Alters the Antigen Phenotype of Human Tumor Cells. J.W. Greiner, F. Guadagni, and S. Pestka. Lab. Tumor Immunology and Biology, NCI/NIH, Bethesda, MD 20892, Roche Institute of Molecular Biology, Nutley, NJ, 07110.

Recombinant human leukocyte (clone A) interferon (IFN-αA) induces alterations in the antigen phenotype of human breast and colon carcinoma cells. Treatment with varying amounts of IFN-αA resulted in as much as a 4-5 fold increase in cell surface antigen expression measured by the binding of monoclonal antibodies (MAbs). The IFN-αA-induced enhancement, a) can occur independent of any changes in cell growth, b) is a result of the expression of more antigen per cell, and c) renders the tumor population more antigenically homogeneous by recruiting previously tumor antigen-negative cells to become antigen-positive. For example, cell sorter analysis revealed that after a 24 hr incubation of a colon carcinoma cell line in the presence of 1000 units IFN-αA the overall mean fluorescence intensity as a result of binding of MAb B1.1 to the cell surface carcinoembryonic antigen (CEA) in the population was increased greater than 3-fold and the percent of CEA positive cells rose from 46% to >90%. Preliminary studies utilizing athymic mice bearing human colon tumors showed an increased tumor antigen content in tumor extracts as well as an enhanced binding of a radiolabeled MAb to the tumor cell surface following a regimen of IFN-αA administration. The findings suggest that in both *in vitro* and *in vivo* models IFN-αA can enhance tumor antigen expression and, thus, augment the binding of a MAb. This biological activity of IFN-αA could conceivably be exploited by designing clinical protocols aimed at using IFN-αA as an adjuvant to enhance the efficacy of conjugated MAbs for tumor detection and/or therapy.

M24 SPECIES SPECIFICITY OF HUMAN AND MURINE α-IFNS IS DETERMINED BY THE AMINO ACIDS SEQUENCE, N.B.K. Raj, R. Israeli, K.A. Kelley, M. Kellum and P.M. Pitha-Rowe, The Johns Hopkins Univ. Oncology Ctr., Baltimore, MD 21205

Comparison of human and murine α-IFNs shows a high degree of similarity with the conservation of 50 of the total 166 amino acid residue positions; yet they are species specific with respect to their biological antiviral activities. In order to dissect the species specificity, a series of novel hybrid interferon genes have been constructed, replacing certain structural regions of human α-IFNs with equivalent regions from murine α-IFNs and vice versa. Replacing the carboxy-terminal 44-45 amino acids of human IFNs with murine α-IFNs, where the hybrid IFNs differ from the cognate human IFNs by 16-17 amino acids, resulted in 10-100 fold increase in their antiviral activities on heterologous mouse cells, while their activities on human and bovine cells are not altered. However, similar modifications to murine α-IFNs by the human α-IFN sequences reduced the activities on murine cells by 100-fold, but showed no activity on bovine or human cells. Hybrid peptides containing only 62 amino-terminal or middle portion from 63-121 amino acids of human IFN had 100-fold less activity on human cells, while the hybrid that contained amino-terminal 62 amino acids and carboxy-terminal 44 amino acids of human IFN showed 10-fold less activity on human cells. The activity of human IFN on bovine cells is decreased by 100-fold when the middle 63-121 amino acids are replaced by murine IFN, while replacing amino or carboxy-terminal portion by murine IFN decreased the activity by 10-fold. The data comparing the antiviral activities of the hybrid peptides and their neutralizing activities with polyclonal antibodies on heterologous cells will be discussed.

M25 DISSOCIATION OF ANTIVIRAL AND ANTICELLULAR ACTIVITIES OF INTERFERONS, Joyce Taylor-Papadimitriou, Nicolette Ebsworth, Franni R Balkwill, David Leavesley and Enrique Rozengurt, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX

Quiescent fibroblasts synchronized in G_0 provide a useful model system for studying the effects of IFN on the induction of cell proliferation by mitogens, as compared to its effect on virus growth. We have observed that quiescent mouse Swiss 3T3 cells or human fibroblasts treated with interferon for only 2 hours before the addition of serum are effectively protected against virus infection for up to 24 hours after the addition of serum and the removal of interferon. In sharp contrast, exposure of quiescent fibroblasts to interferon only before addition of serum or the peptides epidermal growth factor and insulin, has no inhibitory effect on cell proliferation. Further, even when interferon is added after the addition of growth factors, an exposure of several hours is required to inhibit stimulation of DNA synthesis. The data suggest that there are major differences in the mechanisms underlying the antigrowth and antiviral effects of interferon. One conclusion that can be drawn is that certain molecular events induced by interferon treatment of quiescent cells, such as the induction of 2'5' oligoadenylate synthetase are not sufficient to inhibit cell growth.

Although an exposure of several hours is required for an effect on DNA synthesis the IFN can be added up to 6 hours after the mitogens and still be inhibitory. The kinetics for establishment by IFN of an inhibitory effect on DNA synthesis are similar to those shown by tubulin disrupting agents in stimulating DNA synthesis in 3T3 cells. This suggests that an effect on the tubulin network, opposite to that of colchicine could be involved in IFNs growth inhibitory effect. Whether this is so or not, the presence of an intact tubulin network is crucial for the generation of a signal leading to growth inhibition, since tubulin disrupting agents specifically antagonise IFN's inhibitory effect on DNA synthesis. The fact that interferon is effective in inhibiting cell growth when added several hours after mitogens implies that inhibition by IFN of early events stimulated by mitogens (e.g. induction of fos and myc oncogenes and of ODC activity) is not crucial to the inhibitory effect of IFN on DNA synthesis. The dissociation of the antiviral and antigrowth effects in quiescent fibroblasts could be due to differences at the level of receptor interactions, transduction of signals or in the intracellular signals generated. The requirement for a long exposure to interferon for effective growth inhibition, coupled with the fact that the levels of interferon required for this effect are usually much higher than those necessary for the establishment of an antiviral state, suggest that the receptor interactions leading to the antiviral and antigrowth effects may not be identical. It seems likely that for an effective antigrowth effect a higher fractional occupancy of receptors is required and an interferon-receptor complex possibly interacting with cytoskeletal elements must be maintained at the membrane.

Pharmacology of the Interferons

M26 EARLY CLINICAL TRIALS OF INTERFERON-GAMMA, Stephen A. Sherwin, M.D., Department of Clinical Research, Genentech, Inc., South San Francisco, CA, 94080, U.S.A.

Since 1983, more than 1000 patients worldwide have been treated in clinical trials of Genentech's recombinant human interferon-gamma (rIFN-γ). This testing began with phase I safety and pharmacokinetic studies in patients with advanced malignancy and helped define safe treatment regimens for phase II testing in malignant and non-malignant disease. Different methods of adminstration of rIFN-γ were explored in phase I testing and the basic pharmacokinetic properties of the agent were explored (1-4). These trials, which will be reviewed in this presentation, also revealed objective evidence of antitumor activity and immunomodulatory effects. Subsequent to phase I testing, a series of phase II trials were initiated that were intended to further define the anticancer and anti-infective activity of rIFN-γ. Objective evidence of the antitumor and antiviral effects have been observed in these studies. In addition, further indication of in vivo immunomodulatory effects including macrophage activation has been noted (5). Additional studies have subsequently been initiated to explore the anticancer effects of rIFN-γ in combination with other interferons and cytotoxic chemotherapy agents.

1. Kurzrock, R, Rosenblum, M.G., Sherwin, S.A., Rios, A., Talpaz, M., Quesada, J.R., and Gutterman, J. Recombinant gamma interferon: pharmacokinetics single-dose tolerance and biological activity in cancer patients. Cancer Res., 45:2866-2872, 1985.
2. Foon, K.A., Maluish, A., Sherwin, S.A., Abrams, P.G., Holmes, P., Herberman, R.B., Oldham, R.K. Phase I trial of recombinant gamma interferon in patients with cancer. Cancer Immunol. Immunother. (In press).
3. Vadhan-Raj, S., Nathan, C.F., Sherwin, S.A., Oettgen, H.F., Krown, S.E. A Phase I trial of recombinant interferon gamma by 1 hour intravenous infusion. Cancer Treatment Reports. (In press).
4. Vadhan-Raj, S., Al-Katib, A., Nathan, C., Ghalla, R., Pelus, L., Sherwin, S.A., Oettgen, H.F., Krown, S.E. Phase I trial of recombinant interferon gamma in cancer patients J.Clin.Onc. (In press).
5. Nathan, C.F., Horwitz, C.R., de la Harpe,J., Vadhan-Raj, S., Sherwin, S.A., Oettgen, H.F., Krown, S.E. Administration of recombinant interferon-γ to cancer patients enhances monocyte secretion to hydrogen peroxide. Proc.Natl.Acad.Sci. (In press).

M27 CLINICAL EXPERIENCE WITH INTRON A-RECOMBINANT ALFA-2b INTERFERON Robert J. Spiegel, M.D., Department of Oncology Clinical Research, Schering Corporation, Kenilworth, NJ, USA 07033

Clinical trials with INTRON have now been completed in over 1,500 cancer patients. Although alpha interferon alone has not shown broad anti-tumor activity in advanced malignancies, it had shown dramatic activity in almost all patients with a single malignancy, hairy cell leukemia, and has shown high response rates in non-Hodgkin's lymphoma, mycosis fungoides, T-cell cutaneous lymphomas, and Kaposi's sarcoma. Additionally, important activity has been indentified in multiple myeloma, malignant melanoma, and renal cell carcinoma. Other areas which show promising results are loco-regional therapy of superficial bladder cancer and ovarian cancer.

Dose limiting toxicity has been constitutional symptoms including fever and fatigue. These occur to some degree in almost all patients, however, rarely lead to treatment discontinuation. Other side-effects include mild leucopenia and thrombocytopenia which are rapidly reversible. Occasional confusion (primarily in older patients) and transient hepatic enzyme elevations are also notable, although both are readily reversible upon drug discontinuation. Treatment has been well tolerated with few other serious side-effects.

In addition to identifying areas of true activity these trials have suggested some unanticipated principles for the development and testing of other interferons and other biologicals. These include (1) dose and schedule dependency, (2) prognostic significance of prior therapy, (3) prognostic significance of tumor burden, (4) the importance of identifying specific sub-populations that might be peculiarly sensitive to interferon. Results also suggest that IFN and other biologicals might be most active in the adjuvant setting. Additionally, the quality of response (e.g., CR's of long duration) in small numbers of patients may be as important as overall response rates in identifying the true utility of these agents. Studies have begun to assess interferon receptors as predictors of responsiveness. Future research areas to be pursued include combinations of INTRON with other lymphokines, other chemotherapy and radiation therapy.

Inhibitors of Growth and Transformation (Joint)

M28 REGULATION OF HUMAN ras ONCOGENE EXPRESSION BY INTERFERON, Robert M. Friedman, Esther H. Chang, Dvorit Samid, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20902-4799

Interferon (IFN) has several effects on expression of ras. Treatment with IFN of NIH 3T3 that had been transfected with the EJ/T24 c-Ha-ras1, v ras, or v-mos oncogenes inhibited the subsequent development transformed foci. This was observed even when the IFN was added as late as seven days after transfection with the oncs. Similar treatment with IFN had no significant effect on biochemical transformation induced by transfection with the Ecogpt or neo genes. We are currently studying the mechanism of the effect of IFN on transfection with EJ/T24 ras DNA.

IFN was also effective in inducing morphological reversion in NIH 3T3 cells already transformed by an Ha-MuSV LTR-activated c-Ha-ras1 protooncogene (cell line RS485). In these studies treatment with IFN resulted in emergene of colonies of revertant cells in RS 485 cultures. These colonies could be isolated to form revertant clonal lines such as 4C3. Unlike RS 485, revertants grew to low saturation densities in monolayer cultures, did not form foci in soft agar, and did not form rapidly growing tumors after transplantation into nude mice.

While the transforming ras DNA was still present in revertants, unchanged in quantity and distribution, ras mRNA and ras-specified p21 protein were reduced four to eight-fold in IFN-treated 4C3 as compared to RS 485. IFN appeared to inhibit the formation of ras mRNA at a transcriptional level.

When IFN was omitted from 4C3 cultures, retransformant foci appeared; however, these foci formed on a monolayer of untransformed cells. The latter could be isolated to give rise to cell lines in which the revertant state persisted in the absence of IFN treatment (PRs).

The level of p21 and ras mRNA in PRs were comparable to those found in RS 485 cultures, even though the PR cultures behaved biologically like untransformed cells. PRs resisted morphological transformation by EJ/T24 ras transforming DNA, but not biochemical transformation by the neo gene. PRs also resisted transformation by a variety of onc-bearing sarcoma viruses.

While the revertant status of PRs was stable for at least 40 generations after cloning, all of the cells in a PR culture could be retransformed within 6 days of the addition of azacytidine (2.5 mM) .

M29 INHIBITION OF c-myc EXPRESSION AND CELLULAR PROLIFERATION BY ENDOGENOUS AND EXOGENOUS INTERFERONS, Adi Kimchi, Miriam Einat, Dalia Resnitzky, Anat Yarden and Galia Gat, Department of Virology, The Weizmann Institute of Science, Rehovot 76100, Israel

Based on studies performed on in vitro systems of hematopoietic cell differentiation we established that an autocrine mechanism of growth inhibition controls the progressive arrest of differentiating cells in the Go/G1 phase of the cell cycle. We have reported in the past that erythroid and macrophage cell lines produce β-related interferon (IFN) at a certain stage following induction of differentiation by Me_2SO or TPA. By using specific antibodies directed against IFN-β or the IFN type I cell surface receptors we have demonstrated that this factor operates in a typical autocrine mode of action. The expression of two members of the IFN induced gene family, the (2'-5') oligoadenylate synthetase and class I HLA genes, was studied in detail in these systems. Interestingly, the kinetics and extent of self induction of genes in the autocrine system differ from the classical process of gene induction by externally added IFN. Similar endogenous IFN-β is produced and activates gene expression when non immortilized bone marrow-cells are induced to differentiate by colony stimulating factor-1 (CSF-1). In mouse myeloid M1 cells induced to differentiate by CSF-GM we show that antibodies against IFN-β prevent the major part of the reduced proliferation activity of cells. The neutralizing antibodies also abolish part of the reduction of c-myc mRNA levels which takes place during the differentiation process. These data indicate that the autocrine IFN controls the decline in c-myc expression and in growth kinetics during terminal differentiation.

In some cell lines such as Daudi Burkitt lymphoma and Molt-4 cells, exogenous IFN reduces the c-myc mRNA steady-state levels as a result of transcriptional inhibition. The two c-myc mRNA initiated from different promoter regions are equally inhibited and no significant effect of IFN on the stability of the mature transcripts is detected. In many other cell lines, termed as 'resistant', IFN fails to reduce c-myc mRNA although these cells express functional cell surface receptors to IFN. Analysis of the response to IFN of somatic cell hybrids prepared from sensitive and resistant cells indicates that the sensitive phenotype is dominant and suggests that c-myc inhibition could be mediated by IFN induced trans-acting protein elements.

In Balb/c 3T3 fibroblasts the IFN inhibits most of the PDGF mediated increase of c-myc, ornithine decarboxylase and β actin mRNAs as well as part of the increase in c-fos mRNA without impairing the binding of PDGF to its cell surface receptors. We have introduced a deregulated c-myc gene into sensitive Balb/c 3T3 cells using constructions which link the two coding c-myc exons to viral promoters. The c-myc transfected stable clones expressed novel transcripts which were not reduced by IFN. Interestingly, these cells have lost the antigrowth sensitivity to IFN during the Go/G1 to S transition triggered by PDGF or FGF. Reduction of c-myc expression by IFN therefore generates an early restriction in the Go/G1 phase of the cell cycle.

Immunoregulation, Receptors and Clinical Studies

M30 Human alpha and gamma interferons have B Cell Growth Factor (BCGF) activity

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E. coli derived recombinant interferons α_1 , α_2 , α_4 , α_7 and γ (kindly provided by Drs P. Trotta and T. Nagabhushan,, Schering Corporation) were tested for BCGF activity on highly purified human B lymphocytes. The IFN's were tested in 1) their costimulatory effects on B cells suboptimally activated with anti-IgM antibodies and 2) their ability to sustain the proliferation of B cells preactivated with Staphylococcus aureus Cowan (SAC). All the IFN's tested showed a potent transient BCGF activity on anti IgM activated B cells. The IFN's were active over a wide range of concentrations. Blood B lymphocytes could be stimulated with as low as 1 U/ml γ IFN. Non preactivated B cells were not stimulated significantly. In contrast to rec. IL2 and T cell derived BCGF the IFN's were not able to induce the proliferation of SAC preactivated B cells. A rec. γ IFN lacking the Cys-Tyr-Cys AA at the N terminus was as effective as the complete molecule. However, another γ IFN with a 15 AA deletion at the COOH terminus had only very low BCGF activity. The BCGF activity of γ IFN was specific since B cell proliferation was inhibited by an anti γ IFN Mab. In addition α IFN's induced immunoglobulin production by SAC activated B cells, whereas γ IFN did not have such an effect. These results indicate that alpha and gamma IFN's have transient BCGF activity but only α IFN's have B cell differentiation factor (BCDF) activity.

M31 EFFECT OF IFN-γ AND FORSKOLIN ON B-LYMPHOID CELLS

Heidi Kiil Blomhoff[1], Erik Ruud[1], Steinar Funderud[1], Trine Bjøro[2], Susan Pfeifer Ohlsson[1], Rolf Ohlsson[1], Tore Godal[1].
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γ-Interferon (IFN-γ) has been implicated as regulator of cell proliferation as well as expression of MHC antigens in a variety of cells. The mechanisms underlying these effects are however poorly understood. Several investigators have suggested that cAMP functions as a mediator of IFN-action. Therefore, we wanted to compare the effects of IFN-γ and the adenylate cyclase activator, forskolin. We have previously reported that IFN-γ reduced the proliferation-potential in B-precursor cell-lines, with no marked effects on more mature B-cell lineages. In contrast, in both the B-cell precursors and the mature B cells, induction of class I MHC expression was enhanced (1). By using these cell-systems we have addressed the possible similarities in IFN-γ and forskolin function. Thus, forskolin increased the levels of cAMP in the cells severalfold, correlating with a reduction in the proliferation-potential of mature B cells as well as B-cell precursors. Further, in contrast to IFN-γ, forskolin increased the levels of both class I and class II MHC antigens of the B-lymphoid cells, indicating that regulation of the cAMP levels is not an important step in the IFN-γ effect on these cells. We have also examined the effect of IFN-γ and forskolin on regulation of c-myc gene expression. C-myc gene products have been implicated as key controlling elements in normal and malignant cell growth. Interestingly, there were disparate effects of forskolin and IFN-γ on c-myc RNA steady state levels, although both agents inhibit cell proliferation. We are currently studying these effects in more detail, and the results will be presented (1. Blomhoff et al 1985 Scand.J.Imm (in press).

M32 **EFFECTS OF INTERFERONS AND INTERLEUKIN-2 ON TRYPTOPHAN METABOLISM IN HUMANS.**

R.R. Brown, E.C. Borden, P.M. Sondel, P.C. Kohler, G.I. Byrne, C.M. Lee, J.C. Nunnick, J. Schiller and L. K. Lehman. Dept. Human Oncology and Dept. Medical Microbiology. Univ. of Wisconsin Center for Health Sciences, Madison, WI 53792.

The biological response modifiers (BRM), interferons (IFN) and interleukins (IL) offer considerable promise as anti-tumor agents. Although IFNs affect various immune functions, these immune effects can be dissociated from antiproliferative effects. Hayaishi's group showed in mice that virus infections, bacterial lipopolysaccharide treatment, or IFN administration caused marked induction of the tryptophan-degrading enzyme, indoleamine dioxygenase (IDO). Consequently we have assayed serum tryptophan (T), and urinary kynurenine (K) (a major T metabolite) in patients undergoing clinical trials with IFN-alpha, IFN-gamma, and IL-2. Comparison of pre-treatment levels with post-treatment levels showed that IFN-gamma and IL-2 caused a more than 50% decrease in serum T levels and a corresponding increase in urinary K levels. IFN-alpha or beta treatment did not cause such changes. These data support and extend other studies from these laboratories showing that T conversion to K was markedly induced by IFN-gamma (but not by IFN-alpha or beta) in human cell lines in tissue culture. It is tempting to propose that the decrease in serum T (and presumably decreases in cellular T, 5-hydroxytryptophan and serotonin) may be an important part of the anti-proliferative mechanism of these BRMs, and may also relate to clinical side reactions. (Supported in part by NIH-NCI grant ST 32 CA09451).

M33 MHC CLASS II ANTIGENS ON NORMAL AND MALIGNANT RAT ORAL KERATINOCYTES AND THEIR STIMULATION IN VITRO BY INTERFERON GAMMA, Isabel J. Crane, A. Pitigala-Arachchi, C. Scully and S. S. Prime, University of Bristol, Bristol, BS1 2LY, U.K.

The rat model of oral carcinogenesis enables more controlled investigation into the pathogenesis of oral malignant epithelial lesions. This study examines the expression of MHC Class II antigens during the development of oral carcinoma in vivo and in normal and malignant oral keratinocytes in vitro. Oral carcinomas of the tongue and palate were induced in Sprague-Dawley male rats using the carcinogen 4-Nitroquinoline N-oxide. Carcinomas, test tissues taken throughout the experimental period (9 months) and untreated control tissues were divided and half snap frozen in liquid Nitrogen and the remainder cultured using 3T3 fibroblast support. In tissue sections, Class II antigen expression increased throughout the experimental period, associated with an increase in Langerhans cells and also in the later stages of tumour development, with keratinocytes. Cell cultures of both normal and malignant keratinocytes stained positively for keratin but malignant cultures had a greater heterogeneity of cell size and shape, an increased growth rate and capacity for serial cultivation and reduced growth regulation. Our experiments show that MHC Class II antigens are induced on normal and malignant keratinocytes in vitro by the addition of recombinant rat interferon gamma (IFN-γ) to the culture medium and this is dose and time-dependent. The results of this study suggest that the expression of MHC Class II antigens on keratinocytes during the induction of oral carcinoma in vivo may be mediated by IFN-γ released by infiltrating T-lymphocytes.

This work was supported by the Cancer Research Campaign.

M34 SMALL AMOUNTS OF INTERFERON-GAMMA INHIBIT TUMOR GROWTH BY ELICITING HOST SYSTEMIC IMMUNOREACTIVITY, Guido Forni, Annunciata Vecchi, Marco Forni and Mirella Giovarelli, Istituto di Microbiologia, Universita' di Torino, Via Santena 9, 10126 Torino, Italy.

Ten international units (IU) of recombinant (r-) or natural (n-) murine interferon (MuIFN-) gamma were used for the in vivo immunotheraphy of a chemically induced fibrosarcoma (CE-2) of BALB/c mice. In vitro doses of r-MuIFN-gamma below 100 units have a marginal antiproliferative effect on CE-2 cells and do not induce the expression of $H\text{-}2^d$ classII, whereas they increase that of class I products of the major histocompatibility complex. In vivo ten daily injections of 10 IU of r- or n-MuIFN-gamma at the challenge site provide a significant protection against increasing doses of CE-2 tumor cells . This protection was enhanced when non-reactive T-lymphocytes from CE-2 tumor bearing mice were admixed at 10:1 ratio with the CE-2 tumor cells. The combined lymphocyte and r-MuIFN-gamma treatment also inhibited the growth of already established tumors when started before they reached 5 mm mean diameter. Tumor inhibition depends upon the activation of host immune system. The antitumor activity of r-MuIFN-gamma and T-lymphocytes was null , in fact, when mice were first irradiated with 450 rads. Moreover, host leukocytes massively infiltrated the area of tumor growth and the small doses of r-MuIFN-gamma injected activated various host immunoreactivity mechanisms.

M35 FIBRONECTIN ACTS SYNERGISTICALLY WITH INTERFERON, MODULATES TUMORIGENICITY OF HUMAN HEPATOCELLULAR CARCINOMA PLC/PRF/5 IN ATHYMIC MICE. Anwar A. Hakim. Loyola University Medical Center. Maywood. Illinois 60153.

Fibronectin modulates the growth promoting activities of "Human Fibroblast Growth Promoting Factor-HFGPF" on malignant melanoma cells and their cloned hybrid cells(Hakim, Fed. Amer. Soc. Expt. Biol. Fed. Proc. 40: 1816, 1981). The present studies examined the effect of fibronectin (Fn) on tumorigenicity of human hepatocellular carcinoma PLC/PRF/c cells. This cell line produces hepatitis B virus surface antigen (HBsAg) similar in size, morphology and polypeptide composition to the form that occurs in the serum of infected individuals. The hepatitis B viral DNA is integrated into the genome of the PLC/PRF/5 cells, and the cells express three RNA molecules containing specific sequence of HBV. One of these RNAs codes for HBsAg. When implanted subcutaneously into Nu/Nu athymic mice, $1x10^3$ cells produced tumors that killed the animal within 14 days. In vitro, PLC/PRF/5 cells grow as cell suspension that attains confluency in 94 hrs. Increased intra-cellular level cAMP increased the cellular tumoriginicity which was accompanied with increased DNA synthesis. The PLC/PRF/5 levels of cyclic nucleotide were increased by a variety of ligands that stimulate adenylate cyclase, and included cholera toxin, adenosine agonists or cAMP-derivatives. Agents that disrupt the tubulin net work in PLC/PRF/5 act synergistically with cAMP-elevating agents or HFGPF stimulated DNA synthesis and cellular tumorigenicity. Huma plasma fibronectin, either in the culture medium or plated onto the surface of the culture plates, alone or in presence of interferon reduced cellular cAMP and tumorigenicity. The fibronectin is acting as a substrate for cAMP mediated phosphorylation reactions,thus stabilizing and protecting the cytoskeletal elements.

M36 NATURAL KILLER CELLS TREATED WITH AN ALPHA INTERFERON WHICH IS DEFICIENT IN BOOSTING ACTIVITY MANIFESTS AN ALTERED PATTERN OF INTERFERON-INDUCED PROTEINS, Ruth E. Herz and John R. Ortaldo*, Roche Inst., Nutley, N.J. 07110. *Nat'l. Cancer Inst., Frederick, MD. 21701.

Interferons (IFN) exert their effects; immunomodulatory, antiproliferative and antiviral, through the induction of specific proteins. One subtype, IFN-αJ, is deficient in boosting activity of natural killer (NK) cells, but has the other effects. Boosting activity of NK cells after a two hour incubation with numerous IFN-α subtypes, except IFN-αJ, has been demonstrated (Ortaldo et al., Proc. Nat'l. Acad. Sci. USA 81:4926, 1984). This lack of activity might be explained by the inhibited synthesis of an IFN-induced protein. If this is the case, we might determine which protein is involved in boosting activity. Synthesis of IFN-induced proteins in human NK cells treated with IFN-αJ has been compared with synthesis of proteins induced by one, IFN-αA, that has potent activity. Cells treated with IFN-αA show increased synthesis of five proteins. These proteins are not synthesized, or show reduced synthesis, in cells treated with IFN-αJ. Synthesis of these proteins was demonstrated by in vivo labeling of cells with ^{35}S-methionine and separation of proteins by two-dimensional gel electrophoresis. Their apparent molecular weights (kDa) and isoelectric points (in parentheses) are as follows: 80 (6.4), 59 (6.5-6.7), 56 (7.0), 49 (6.8) and 63 (5.7).

M37 Suppression of Natural Killer (NK) Cells by A Synthetic Peptide Homologous to Retroviral p15E. H.S. Koren+, D.T. Harris°, G.J. Cianciolo* and R. Snyderman*, +EPA, Clinical Research Branch, Chapel Hill, N.C., °Center for Environmental Medicine, UNC, Chapel Hill, N.C. and *Duke Medical Center, Durham, NC.

It has previously been shown that the retroviral protein p15E is immunosuppressive to monocyte and lymphocyte functions. Here, we describe the effect on NK activity of a synthetic peptide (CKS-17) with homology to a conserved region of p15E. Enriched human NK cells were assayed against K562 tumor targets in a 2h ^{51}Cr release assay. Pretreatment of NK cells with CKS-17 (ca. 5μM), but not with the control peptide neurotensin or BSA at the same concentrations, markedly and reproducibly suppressed NK activity (50-80% inhibition). Significant inhibition was still demonstrable at 1.2 μM dilution of CKS-17. Prior exposure of NK cells to interferon α (IFNα) at 10^3 U/ml did not alter their sensitivity to CKS-17. In contrast, pretreating NK cells with CKS-17 almost entirely diminished their responsiveness to IFNα. Kinetics experiments demonstrated that CKS-17 suppresion of either endogenous or activated NK cells was reversible after 18h at 37°C. Conjugate experiments at the single cell level confirmed the ^{51}Cr release data and further revealed that CKS-17 interferes with the lytic and not with the binding phase. Taken together the data indicate that CKS-17 a synthetic peptide homologous to the immunosuppressive retroviral envelope protein p15E suppresses not only monocyte and T cell functions but also NK activity.

M38 BINDING OF NATURAL MURINE 125I-INTERFERON-γ TO MOUSE CELL RECEPTORS. Santo Landolfo, Franca Cofano, Antonella Fassio and Giorgio Cavallo. Institute of Microbiology, University of Torino, 10126 - Torino, Italy.

Natural murine interferon-γ (naMuIFN-γ), produced by the T lymphoma L12R4 stimulated with phorbol myristate acetate, purified on an anti-MuIFN-γ immunoadsorbent, was labeled with 125-I to study its binding to mouse cell receptors. All the cell lines examined bound 125-I, although the binding varied considerably among the various cell lines. In competition binding experiments, unlabeled natural and recombinant MuIFN-γ, but not murine IFN-α, murine IFNβ or human IFN-γ competed with 125I-IFN-γ for receptor binding on mouse cells. The KD determined by adding increasing concentrations of unlabeled naMuIFN-γ to binding assays varied from 2 x 10(-10) to 2 x 10(-11)M depending on the cell line, with a number of receptors between 500 and 5000 binding sites. Pretreatment of the target cells with proteases but not with endoglycosidases prevented 125I-IFN-γ binding suggesting that the binding site has a protein moie ty involved in the interaction with the ligand. The turnover of the IFN-γ receptor measured by inhibiting protein synthesis with cycloeximide or puromycin was found to have an half life of about two hours. Finally incubation of 125I-IFN-γ with anti-MuIFN-γ monoclonal antibodies prevented the binding of labeled IFN-γ suggesting a close homology between anti-IFN-γ antibodies and IFN-γ binding sites. (Supported by the Italian National Researc Council, PFCMI).

M39 REGULATION OF INTERLEUKIN-2 RECEPTOR EXPRESSION IN HUMAN T-CELLS DURING INTERFERON TREATMENT, Erik Lundgren, Bengt Friedrich, Anders Berg and Martin Gullberg, Inst. for Applied Cell and Molecular Biology, Univ. of Umeå, S-901 87 Umeå, Sweden.

Human anti-T3 activated T-cells were synchronized into the G_0/G_1 phase of the cell cycle by interleukin-2 (IL-2) deprivation. IL-2 receptor positive cells in G_0/G_1 expressed normal amounts of high affinity IL-2 receptor, while the level of the Tac antigen was low. IL-2 induced reexpression of the Tac antigen, together with reentry into the cell cycle, were studied in the presence of rIFN-α_2.

At doses inhibitory to growth a dose dependent inhibition of reexpression of the Tac sites was observed. The effect was sustained and followed during 6 days.

The growth inhibition achieved by IFN was possible to block by neutralizing antibody and the cells were shown to carry IFN receptors at numbers comparable to that of Daudi cells.

A model for the role of IL-2 receptor turnover in modulation of T-cell growth inhibition by IFN will be presented.

M40 THE EFFECT OF αcr-INTERFERON ON MULTIPOTENT PRECURSORS FROM HAIRY CELL LEUKEMIA PATIENTS. R. Michalevicz, D. Aderka, Ichilov Hospital, Tel-Aviv & M. Revel, Weizman Institute, Israel.

The aim of the present study was two fold a) to grow multipotent precursors (CFU-GEMM assay) in peripheral blood and bone marrow of Hairy cell (HC) leukemic patients as compared to normals, b) to analyze the effects of αcr-interferon (Inter-Yeda Ltd, Israel) added at day 0 to the cells for 14 d. It was found that in Hairy cell leukemia patients there is a marked decrease in growth of multipotent precursors as compared to normal controls ($p < 0,001$). Also, colonies from HC were grown which contained lymphoid and myeloid cells. Evidence for clonality was provided both by linearity of growth and also by following the formation of clones at various days. Those lymphomyeloid precursors were stained with B-cell markers. Interferon did not significantly effect the number of colonies at 10 and 100 U but promoted a change in the composition of clones. It was found that when interferon is added to cultures, more myeloid and monocytic cells are present in the colonies while the number of lymphocytes decreases significantly ($p < 0.05$). This was demonstrated by analyzing all the colonies grown with and without interferon. Thus, αcr Interferon seems to induce granulocytic macrophage differentiation in bipotent lympho-myeloid precursors grown from Hairy cell leukemia patients. The described in vitro effect in HC precursors may provide an explanation for the in vivo response of HC patients to αcr-Interferon.

M41 MODULATION OF THE RESPONSE OF CML CFU-GM TO GROWTH REGULATION FOLLOWING TREATMENT WITH RECOMBINANT HUMAN GAMMA INTERFERON (HR-IFNg). A PHASE I TRIAL. Louis M. Pelus, S.Vadhan-Raj. Sloan Kettering Inst., N.Y.,N.Y. 10021.

Culture studies and animal experiments indicate that the proliferation of granulocyte-macrophage progenitor cells (CFU-GM) is regulated by prostaglandin E (PGE) and acidic isoferritins (AIF). These effects are associated with CFU-GM cell cycle related expression of HLA-DR. Agar culture studies demonstrate inhibition of normal DR+ CFU-GM by PGE (ID50 10^{-7}) and AIF (ID50 10^{-11}). Marrow CFU-GM from patients with CML are hyporesponsive to PGE (ID50 $>10^{-5}$M) and display diminished levels of DR (N 50±6%;CML 17±6%, n=25). These parameters were evaluated in a patient with PH1+ CML in chronic phase treated with HR-IFNg. The patient was treated with 10^7U/m^2/day by 6 hr infusion 5 days/wk for 2 wks. This was followed by a 2 wk rest, a second 2 wk cycle and maintenance with HR-IFNg at the same dose, im, daily. Prior to treatment CFU-GM were unresposive to PGE and AIF and displayed no HLA-DR on CFU-GM (5±2% DR+). By 16 days post-IFN culture studies demonstrated normalized response to PGE, AIF and HLA-DR expression. All metaphases persisted as 9+,22q$^-$,Ph1. These results were observed on serial examination for 5 mo. At 5 mo post-IFN CFU-GM response to PGE and AIF as well as DR expression reverted to hyporesponsive. This was coincident with appearance of a 3-x,22q$^-$ clone (62%). By 7 mo post IFN this clone expanded to 88%. Treatment was discontinued and the patient received an allogeneic marrow transplant. At 4 mo post transplant CFU-GM response to PGE and AIF, and DR expression were normal. These findings indicate that treatment with HR-IFNg can modulate CML CFU-GM growth characteristics and provide further evidence for the importance of HLA-DR antigen in the regulation of CFU-GM proliferation by PGE and AIF.

M42 QUANTIFICATION AND CHARACTERIZATION OF HIGH AFFINITY MEMBRANE RECEPTORS SPECIFIC FOR TUMOR NECROSIS FACTOR (TNF) ON HUMAN CELL LINES, Peter Scheurich, Ugur Ücer, Martin Krönke and Klaus Pfizenmaier, Clinical Research Group BRWTI, Max-Planck-Society, Goßlerstr. 10d, 3400 Göttingen

The expression of specific membrane receptors for tumor necrosis factor (TNF) was determined on various human leukemic cell lines differing in their sensitivity to the growth inhibitory activity of TNF. Binding studies with ^{125}I-labelled recombinant human TNF revealed specific, saturable binding of TNF with a dissociation constant of ~ 1 x 10^{-10} M. Scatchard analyses revealed approximately 3370 and 2560 TNF-specific binding sites/cell on K562 and U937 cell lines, respectively. Disuccinimidyl-suberate-crosslinking of ^{125}I-TNF bound to these cell lines and SDS-PAGE of membrane extracts revealed a single receptor protein with an apparent molecular weight of 76,000 Dalton. Interaction with specific cell surface receptors appears to be required for TNF function, since receptor negative variants of K562 proved TNF resistant. However, some of the cell lines (e. g. Jurkat), which express TNF receptors, were also resistant to TNF, indicating that TNF sensitivity is determined both at the level of receptor expression and at a post-receptor level. Interferon gamma, which potentiates TNF mediated cytotoxicity, was found to modulate TNF receptor expression only slightly. On the other hand, TNF enhances Interferon gamma-induced HLA class I and class II antigen expression in various cell lines, suggesting that TNF acts as a regulator of cellular functions not related to cell growth.

M43 Mutagenesis of BoIFN as a tool for identification of IFNα epitopes interacting with the specific IFNα cell receptor. A. Shafferman and B. Velan, Israel institute for Biological Research, Ness-Ziona 70450, Israel

Leukocyte bovine interferon was (Leu-BoIFN) tested for its ability to induce an antiviral state in various mammalian cell and was found to be specific to cells from bovine origin (1). Five BoIFNα distinct genes were isolated from a bovine genomic library (2). Like Leu-BoIFN, also the different bacterial BoIFNα gene products exhibit antiviral activity which is specific to bovine cells with no detectable activity on human cells . On the basis of a comparative analysis of bovine and different mammalian IFN-α amino acid sequences the potentially functional domains on the molecule were postulated. In-vitro derived mutants at the postulated functional domains on BoIFNα were used to identify the epitope responsible for the cell specific antiviral property of BoIFNα. Analysis of different BoIFNα mutants provides an insight into the IFNα domain interacting with its cell receptor.

1. S. Cohen, B. Velan, T. Bino, H. Rosenberg and A. Shafferman. Methods Enz. Vol. 119. In press.
2. B. Velan, S. Cohen, H. Grosfeld, M. Leitner and A. Shafferman. J. Biol. Chem. 260, 5498 (1985).

M44 AN IMMUNOGENIC REGION OF THE HuIFN-α2 DEFINED BY MONOCLONAL ANTIBODIES Moira Shearer, Joyce Taylor-Papadimitriou, Dorota Griffin and Franni R Balkwill, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

We have developed several monoclonal antibodies to HuIFN-α2. Four of these neutralise the antiviral activity of HuIFN-α2 but do not react with HuIFN-α1. They react with IFNs on Western blots of reducing SDS-PAGE suggesting that the antigenic determinants may be continuous peptide sequences. Using IFN analogues and hybrids, epitope analysis was carried out on these four antibodies and a similar one obtained from Wellcome Laboratories, using an ELISA assay. AAs in the 107-113 region are implicated in the sites recognized by 4 of the 5 antibodies analyzed. Substitutions at 112 and 113 reduce the binding of 3 of the antibodies, 2 of which are also affected by amino and carboxy terminal truncations of HuIFN-α2. The fifth antibody recognizes HuIFN-αs with arginine at position 120 (IFN-α2) but not IFNs with lysine in this position. The pattern of reaction of this antibody with other IFN analogues supports the idea that the arginine at 120 forms part of or affects the conformation of the specific epitope.

M45 BINDING CHARACTERISTICS OF AND MEMBRANE RECEPTORS FOR RECOMBINANT IFNγ ON HUMAN TUMOR CELLS DIFFERING IN IFNγ SENSITIVITY, Ugur Ücer, Christian Ertel, Peter Scheurich and Klaus Pfizenmaier, Clinical Research Group BRWTI, Max-Planck-Society, 3400 Göttingen, F.R.G.

IFNγ action is induced by binding to cell membrane receptors, which are distinct from the binding sites for α/β-Interferons. However, cell lines, which specifically bind IFNγ with high affinity, can be completely resistant to one or the other of the various biological IFNγ effects raising the question of structural and functional heterogeneity of IFN receptors. In order to approach this question, we have investigated tumor cells, which differ in their sensitivity to the antiproliferative effect of IFNγ, and performed receptor crosslinking studies with ^{125}I-labelled IFNγ as well as kinetic studies on the binding and processing of IFNγ by these cells. Our data suggest that in both IFNγ sensitive and in IFNγ resistant cell lines, the IFNγ receptor is a protein complex of approximately 128 kDa, which consists of two subunits of 75 and 53 kDa. Scatchard analysis on a large variety of established tumor cell lines and fresh patient material revealed high affinity binding ($kD \sim 10^{-10}$ M), but a great difference in the number of membrane receptors on distinct cells of the same tissue origin. Further, we obtained evidence that tumor cells resistant to growth inhibition by IFNγ differed from sensitive tumor cells in the processing of cell-bound IFNγ. Thus, resistant cells were capable to rapidly inactivate and release IFNγ from the membrane. SDS PAGE analysis revealed a size reduction of processed IFN, which completely lacked binding activity, of approximately 2 kDa. Further investigations will reveal, whether this inactivation is crucial for the observed resistance to growth inhibition.

M46 GROWTH INHIBITORY MECHANISM OF RECOMBINANTβ INTERFERON ON HUMAN GLIOMA CELLS IN CULTURE. W.K.A. Yung, P.A. Steck, R.P. Moser, and K. Nishioka. U.T.M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030.

Growth inhibitory effect of recombinant α and β interferons on several human glioma cell lines were evaluated by growth assays. A differential sensitivity to recombinant interferon ($IFN\beta_{ser}$) was observed with ID50s ranged from 50 to 500 U/ml. All glioma cell lines tested were resistant to recombinantαinterferon to 2000 U/ml. We have begun investigating possible mechanism of action of $IFN\beta_{ser}$ on these glioma cell lines. Preliminary data has shown that incubation with $IFN\beta_{ser}$ decreased cellular putrescine level significantly when compared with untreated cells. This suggests that $IFN\beta_{ser}$ might exert its growth inhibitory effect by inhibiting Ornithine Decarboxylase (ODC) activity. Since aberrant expression of epidermal growth factor receptor (EGF-R) gene has been recently described in shome human glioblastoma, we have examined the expression of EGF-R on these glioma cell lines. The number of EGF-R was estimated by ^{125} I-EGF binding to be approximately 5×10^4/cell to 1×10^5/cell. Incubation with $IFN\beta_{ser}$ modified the ^{125} I-EGF binding on to these glioma cells suggesting possible modulation of EGF-R expression on human glioma cells by IFN treatment. Further experiments are ongoing to determine the effect of $IFN\beta_{ser}$ on ODC activity as mechanism of growth inhibitory action, and any possible relation between modulation of EGF-R function and growth inhibition by $IFN\beta_{ser}$ on human glioma cells. (Supported by grants NCI CA33027 and from John S. Dunn Research Foundation to W.K.A. Yung).

Interferons and Oncogenes

M47 EFFECT OF INTERFERON ON THE TRANSFORMED AND NORMAL PHENOTYPES OF A MOUSE CELL INFECTED WITH R.S.V. TEMPERATURE SENSITIVE FOR TRANSFORMATION, Gila Arad and Amos Panet, Hebrew University-Hadassah Medical School, Jerusalem 91-010, Israel.

We have studied the effect of Interferon on the regulation of biochemical events linked to the cell cycle of normal and transformed cell cultures. For this purpose we developed a mouse 3T3 cell line infected with a Rous Sarcoma Virus mutant, designated LA90, temperature sensitive for transformation. This mutant virus codes for a thermolabile $pp60^{src}$ protein. Serum deprivation at the nonpermissive temperature arrests the cells in the G_0/G_1 phase of the cell cycle. We measured the induction of ornithine decarboxylase (O.D.C.) activity as a marker for early G_1 phase of the cell cycle. Cells were stimulated to proliferate either by the addition of serum at the nonpermissive temperatue or by shift-down of the cells to the permissive temperature in the absence of serum. Results indicate that cells stimulated to proliferate by shift down to the permissive temperature are more sensitive to the inhibitory effect of IFN than cells stimulated to proliferate by the addition of serum at the nonpermissive temperature. We measured the effect of IFN on the rate of inactivation of O.D.C. We could not observe any change in the rate of O.D.C. inactivation in cells treated with IFN as compared to untreated cells or to cells treated with cycloheximide.
The effect of IFN on the induction of c-fos and c-myc in $pp60^{src}$ activated cells is under investigation.

M48 IFN-INDUCED-PHENOTYPIC CHANGES IN HUMAN TUMOR CELLS IN RELATIVE TO THE EFFECT OF IFN ON ONCOGENE EXPRESSION, Danièle Brouty-Boyé, Janine Wybier-Franqui and Horacio Suarez, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France

Investigations of the effects of IFN-α on the phenotype of human tumor cells showed that long-term IFN treatment induced a partial reversion of the phenotype of osteosarcoma cells, as evidenced by a reduction in cell proliferation, absence of cellular overlapping in confluent cultures and a significant reduction in tumorigenicity. Nevertheless, the transforming ability, genomic level and expression of the activated c-Ki-ras present in these tumor cells were not modified by IFN-treatment. In contrast, long-term IFN treatment did not reverse but even potentiated some of the phenotypic characteristics (including tumorigenicity) of bladder carcinoma and gastric sarcoma cells. In both tumor cell lines, the expression of activated oncogenes, c-Ha-ras and c-N-ras respectively, were unaltered by IFN-treatment. As IFN does not appear to alter oncogene activity, our results suggest that IFN induces cellular changes that modify the cells response to the oncogenic products.

M49 EFFECT OF INTERFERON TREATMENT ON MALIGNANT TRANSFORMATION AND ONCOGENES EXPRESSION IN MAMMALIAN CELLS. I - BIOLOGICAL STUDIES.
Martine Canivet, Gilles Mercier, Fortuna Saal, Sylvie Paulien, J. Valla-Bourébia, Yolande Poirot, Jorge Périès.
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Long term Interferon treatment of murine Kirsten sarcoma virus transformed Balb/c cells (Ki-Balb cells) induces morphological reversion and increases expression of v-Ki-ras oncogene.

In this system, we have studied the modification of some parameters related to transformed phenotype and tried to correlate them to the molecular events. We observed that INF treated cells present :

a) an increased tumoregenicity in conventional and nude Balb/C mice.
b) a higher plating efficiency under conditions of serum depletion.
c) an increased anchorage growth in soft agar with formation of larger colonies.

The effects of long and short term INF treatment were studied in other mammalian cells. Results concerning the interrelationships between molecular and macrobiological events will be discussed.

M50 ENHANCED EXPRESSION OF V-MOS AND α_1(I) COLLAGEN GENES IN MOLONEY SARCOMA VIRUS TRANSFORMED BALB/C CELLS REVERTED TO NON MALIGNANCY AFTER LONG TERM INTERFERON TREATMENT. C.Chany, J.Gerfaux, D.Sergiescu. INSERM U.43, Hôpital St Vincent de Paul, 74, av. Denfert Rochereau 75674 Paris cedex 14.

Continuous treatment for over 1000 generations of Moloney Sarcoma Virus transformed BALB/c fibroblasts with low doses of α/β murine interferon led to the selection of a new non malignant cell population (MSVIF+)(1). Phenotypic reversion could be related to the reconstitution of the cytoskeleton (2) and to the neosynthesis of a dense but faulty extra-cellular matrix (3). Although the MSVIF+ cells did not produce tumors in nude mice, the v-mos oncogene was integrated in the cellular DNA and was transcriptionally active. Helper Murine Leukemia Virus was produced by the cells, but no v-mos containing virions were detected.To investigate the mechanism of phenotypic reversion, the expression of v-mos and that of three genes coding for the extracellular matrix (α_1(I),α_2(I) collagen and fibronectin) were analyzed in parallel.

(1) CHANY C. and VIGNAL M.(1970) J.Gen.Virol.7,203-210.
(2) BOURGEADE MF.,ROUSSET S.,PAULIN D.,CHANY C. (1981) J. Interferon Res. 1,323-332.
(3) GERFAUX J., ROUSSET S., CHANY FOURNIER F., CHANY C.(1981) Cancer Res. 41, 3629-3639.

M51 MACROPHAGE -T LYMPHOCYTE COOPERATION FOR INDUCTION OF GAMMA INTERFERON, Ferdinando Dianzani, and Guido Antonelli, Instit. of Virology, Univ. of Rome, Italy.

Antigenic and mitogenic stimulation of mouse or human lymphocytes for gamma interferon (IFN) production often requires oxidation of galactose residues on the macrophage membrane and activation of calcium flux through the lymphocyte membrane. After mitogenic stimulation macrophages release a soluble factor, different from IL-1, which is capable of stimulating IFN production in T lymphocytes. Taken together these findings raise the possibility of the need for specific antigen recognition by the macrophages for the IFN response. Experiments carried out to test this hypothesis demonstrated that macrophages can effectively cooperate with specifically sensitized lymphocytes only when derived from mice immunized with the same antigen. Taken together the data indicate that macrophages must have some type of specific antigen recognition to initiate the IFN response. Since cooperation by macrophages from immunized animals is abolished by treatment with antibody to murine IgG, and since the ability to cooperate may be conferred on macrophages from unimmunized mice by pretreatment with serum from immunized mice, it is possible that the specific cooperation by immune macrophages is due to membrane bound specific antibody. The possibility that macrophages can exert an antigenically specific function, may provide new approaches to the study of cellular interactions involved in lymphokine production and immune modulation.

M52 REGULATION OF C-MYC AND N-RAS GENES IN DAUDI CELLS BY HUMAN INTERFERON-β, Gerald J. Jonak, Barbara K. Friedland, Elizabeth D. Anton, Ernest Knight, Jr., Central Research and Development Department, E. I. du Pont de Nemours and Company, Wilm., DE 19898

We have analyzed the expression of the c-myc and N-ras genes in Burkitt lymphomas whose growth is regulated differently by human interferon-β (IFN-β). In Daudi cells, whose growth is inhibited by IFN-β, c-myc RNA level is reduced. The c-myc RNA is apparently regulated at a post-transcriptional level because the rate of c-myc RNA synthesis in cell nuclei is not reduced even after 24 hours of IFN-β treatment. N-ras RNA is changed qualitatively and quantitatively in IFN-β treated Daudi cells; in total RNA preparations its electrophoretic mobility is primarily decreased and in the polysomal fraction both its mobility and amount are decreased. We are currently determining, using immunoprecipitation, whether the c-myc and N-ras proteins are regulated in concert with their respective RNA levels. Initial experiments indicate that IFN-β causes a reduction in Daudi cells of the 66,000 and 63,000 c-myc proteins while causing the appearance of a new "c-myc-related" protein of 61,000. Cycloheximide or emetine pre-treatment of IFN-β-treated Daudi cells abrogates the changes in the N-ras but not c-myc gene. In Namalva and Raji cells, whose growth is not reduced with IFN-β, the steady state levels of c-myc and N-ras RNAs are not altered. These results indicate that the c-myc and N-ras gene regulation is not an obligatory response of all cells to IFN-β treatment, but may be related to the decreased proliferative potential of IFN-β-treated Daudi cells. To explore this possibility, we are determining the c-myc and N-ras RNA expression in several sublines of Daudi that differ in their growth response to IFN-β treatment.

M53 INTERFERON INHIBITION OF GENE TRANSDUCTION BY INFECTION WITH AN ADENO-ASSOCIATED VIRUS VECTOR, Catherine A. Laughlin, Uniformed Services University of the Health Sciences, Bethesda, Maryland, 20814.

Adeno-associated virus (AAV) is a ubiquitous defective human parvovirus that efficiently establishes latent infections in human cells by integration of the viral genome into host cell DNA. Superinfection of latently infected cells with a helper herpes or adenovirus results in the rescue and replication of the AAV DNA. Several studies have demonstrated that interferon treatment inhibits the transfer of exogenous genes to mammalian cells by transfection. The evidence suggests that the inhibition is at the level of integration rather than expression of the foreign genes. Transfection is an extremely unnatural process involving large quantities of DNA per cell and illegitimate recombination of the transferred DNA. Therefore it is important to determine whether interferon can inhibit the integration of exogenous DNA under the more natural conditions of viral infection.

Human and mouse cells were treated with α-interferon and then infected with an AAV-based neo vector. G418 selection of the infected cells resulted in a 90% inhibition of colony formation in the interferon-treated cultures. The low levels of interferon used had no detectable effect on cell number or colony forming ability in the presence or absence of G418 in control G418 resistant and sensitive cells. The inhibitory effect of interferon was greatly reduced (0-50% inhibition) when added 36 hr p.i. This finding is consistent with the hypothesis that interferon inhibits integration rather than expression of the transduced gene. Surprisingly interferon did not inhibit the rescue of AAV integrated sequences in latently infected cells following helper virus superinfection.

M54 INDUCTION OF INTERFERON SENSITIVITY IN RESISTANT CELLS BY SPECIFIC GENE SEQUENCES.
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We have shown that a thymidine kinase (tk) deficient strain of mouse L-929 cells is incapable of responding to murine interferons (IFN) by activation of an antiviral state and induction of double-stranded RNA dependent enzymes. Treatment of these Ltk- aprt- cells with IFN does, however, cause an inhibition of cell growth. The ability of IFN to induce antiviral responses is restored in many clones derived by introduction of either a Herpes simplex virus (HSV) or a chicken tk gene, though not by transfection with several other selectable DNA sequences. Further analysis has shown that IFN sensitivity is not directly related to expression of tk enzymatic activity and the capacity of Ltk- aprt- cells to respond to IFN can be restored by introduction of fragments of the HSV tk gene. At least two regions of the coding sequence are able to modify responsiveness to IFN and a homology search suggests a role for two 27 bp sequences separated by 300 bp which are 80% homologous. These sequences contain unique Bal I restriction endonuclease sites and cleavage of cloned HSV tk DNA with this enzyme causes an abrogation of its ability to restore IFN sensitivity when co-transfected into Ltk- aprt- cells with an aminoglycoside phosphotransferase marker gene. Cleavage with Xma III which cuts the tk gene at several sites distant from the repeated sequences does not affect the ability to restore IFN sensitivity. Our results suggest that the sequences identified in some way are able to modulate the capacity of certain genes to be activated by IFN.
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M55 Regulation and Functional Studies of IFN-γ Induced Genes in Human Cells.
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γ-interferon (IFN-γ), a lymphokine secreted by activated T-cells, is a potent activator of the immune state. Genes that are induced by recombinant-IFNγ in human cells are being studied in order to (1) isolate and characterize those molecules that contribute to the IFN-γ response; and (2) use rIFN-γ induced genes as a model system to study cell-surface receptor mediated control of gene expression. Two novel genes that are induced by rIFNγ in the U937 monocyte-like cell-line have been cloned. Interestingly, these genes are also induced in a diverse population of cells including fibroblasts and endothelial cells. One gene has been characterized more extensively (Luster, A.D., Unkeless, J.C. and Ravetch, J.V., Nature 315:672-676, 1985) and has been shown to be an early response gene that is transcriptionally activated by rIFNγ. This gene encodes a protein of relative molecular mass 12,378 which surprisingly has significant amino acid homology to platelet factor-4 and β-thromboglobulin, two chemotatic proteins released by platelets on degranulation. A peptide has been synthesized to the predicted carboxy 22 amino acids and used to raise rabbit serum and isolate monoclonal antibodies. These antibodies recognize this gene product expressed in E. coli cells by Western blotting and are currently being used to identify and purify this molecule from rIFNγ induced U937 cell supernatants. In order to elucidate the molecular mechanisms involved in the regulation of these IFN-γ induced genes, genomic clones have been isolated from λ libraries so that their genomic organization and regulatory elements can be characterized. Transfection experiments are underway to identify IFNγ inducible DNA sequences.

M56 EFFECT OF INTERFERON TREATMENT ON MALIGNANT TRANSFORMATION AND ONCOGENES EXPRESSION IN MAMMALIAN CELLS. II - MOLECULAR STUDIES.
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Studies on oncogene expression in mammalian cell lines treated with human or murine highly purified Interferons were performed. The cell lines examined represent retroviral transformed (S^+L^-) or malignant undifferentiated cells. The interferon-mediated regulation of oncogene expression was observed after short and long term treatment.

The expression of v- and c-oncogenes was studied by dot blot and Northern blot hybridization of poly A RNA. The results were related to the cellular growth, cell cycle distribution and transformation behaviour of the cells (as described in I). In retroviral transformed cells, v-Ki-ras and an endogenous retroviral gene (IAP) were increased 4 and 3 folds respectively as compared to non treated cells. This elevation in Ki-ras expression is selective, as other gene transcripts are diminished or unchanged. For instance, in all the cells examined c-myc expression decreased by about 4 fold. This effect was blocked by IFN antisera. The c-myc down regulation is not directly related to a change in the state of the proto-oncogene methylation.

The results of these analysis will be presented and discussed in the context of the effect of Interferon treatment on the regulation of oncogene expression and malignant behaviour of the cells.

M57 EFFECT OF INTERFERON AND CYCLOHEXIMIDE ON TRANSFORMATION BY TRANSFECTION OF NIH/3T3 INTERFERON-RESISTANT AND SENSITIVE CELL LINES, Samuel Salzberg and Sara Dovarat, Bar-Ilan University, Ramat-Gan, Israel 52100

The effect of IFN on the integration and expression of transformed sequences was studied. NIH/3T3 cells were transfected with high MW DNA extracted from the MSV transformed cell line, clone 124. IFN (500 IU/ml) was either added just before transfection and removed at different times thereafter, or added at different times after transfection and removed 14 days later. Cells were allowed to remain without IFN for 5 days. Virus release and the appearance of the transformed phenotype were then monitored. The following results were obtained: a) Appearance of transformed cells, as well as the release of infectious virus particles, were totally inhibited by IFN when present for 10-14 days after transfection. This inhibition was irreversible. b) Addition of IFN 7 days after transfection yielded only a partial inhibition of either cell transformation or virus release. However, if IFN was added 14 days after transfection, only a minor effect on these parameters was observed. In order to elucidate the mechanism of this IFN effect, we followed the effect of cycloheximide (CH) on the parameters indicated above. Transfected cells were incubated with the drug (0.5 ug/ml) for various time periods and then refed with fresh medium for at least 8 days. Results indicated that 6 days treatment with CH immediately following transfection irreversibly inhibited integration and expression of the examined sequences, whereas 3 day treatment had only a slight effect. We conclude, therefore, that both IFN and CH manifest similar early effects on transfected NIH/3T3 cells, indicating the possibility that a protein whose synthesis is inhibited by these agents may be involved in the integration process. Finally, we could demonstrate that IFN failed to inhibit the transformation of transfected A-10 cells derived from NIH/3T3 and lacking the inducibility of (2'-5') oligoadenylate synthetase activity by IFN.

Gene Regulation and Differentiation

M58 GENETIC VARIATION AND ORGAN SPECIFIC PRODUCTION OF α AND β IFNS IN NDV INFECTED C57BL/6, BALB/cJ AND AKR MICE, Diane Biegel and Paula M. Pitha-Rowe, The Johns Hopkins Univ. Oncology Ctr., Balto., MD 21205

The organ specific expression of α and β interferon genes on the RNA level was studied in NDV infected C57BL/6 and BALB/cJ mice which were previously shown to be high and low producers of circulating interferon. Mice were injected i.p. with NDV, and at various times after infection total and poly(A)$^+$ RNA was isolated from brain, liver, lung and spleen, and analyzed by Northern hybridization with murine α_4 and β_1 IFN riboprobes. Coincident production of α + β mRNA was found localized primarily to the spleens of both strains, although low levels of poly(A)$^+$ message were detected in liver as well. The level of α-IFN was found to be 5-10X higher in C57BL/6 than in BALB/cJ. Additionally, the expression of interferon genes was studied in AKR mice and correlated with the expression of ecotropic and MCF virus in these animals. Constitutive production of large molecular weight transcripts was observed. Furthermore, expression of c-myc and the H-2 genes of the MHC were determined as a function of NDV infection. H-2 was markedly enhanced (20-50X) after 18 hr of infection in all organs tested, while c-myc levels were increased 10-20X in the liver. Thus, it appears the levels of circulating interferon are determined by the rate of transcription and/or differential processing of the transcripts, and both of these events are genetically restricted.

M59 TWO INTERFERON-INDUCED PROTEINS ARE INVOLVED IN THE PROTEIN KINASE COMPLEX DEPENDENT ON DOUBLE-STRANDED RNA, Julien Galabru and Ara G. Hovanessian. Unité d'Oncologie Virale, Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France

The double-stranded (ds)RNA-dependent protein kinase (human) is composed of two subunits : a 68,000-Mr (p68) and a 48,000-Mr (p48) protein. The p48 seems to be responsible for the phosphorylation of the p68 in the presence of dsRNA. The p68 subunit, once it is phosphorylated, becomes a protein kinase capable of phosphorylating exogenous substrates such as eIF2 or calf thymus histone. Both subunits of the protein kinase complex have ATP binding sites and exist in a 110,000- 120,000-Mr protein complex bound by hydrophobic and ionic interactions. The p48 and the p68 are induced by interferon. The isoelectric point (pI) of p48 is 5.8 whereas p68 exists as several subspecies with pIs ranging between 8.2 and 7.4.

Our results indicate that dsRNA-dependent protein kinase is characterized by two distinct protein kinase activities, one functional on the endogenous substrate (the p68 subunit) whereas the other is active on exogenous substrates, eIF2 and histone. Besides the dsRNA, heparin is an efficient activator of the protein kinase complex for the phosphorylation of the p68. The phosphorylation of exogenous substrates is independent of dsRNA or heparin. The dsRNA-dependent protein kinase from mouse L-929 cells is a 110,000-Mr protein complex composed of two subunits with specific ATP binding sites : a 65,000-Mr (p65) and 44,000-Mr (p44). The binding of ATP to the p65 subunit is increased significantly, when the p65 has been saturated with phosphate. By analogy with the human protein kinase therefore, the p65 might be the subunit with kinase activity on the exogenous substrates.

M60 INTERFERON EFFECTS ON CELL DIFFERENTIATION. S.E.Grossberg, J.Taylor, & V.Kushnaryov, Department of Microbiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Cell differentiation is a process of temporal and spatial changes in cellular macromolecular synthesis and composition, resulting in the acquisition of specialized cell function, form, and organization. Early studies showed that the capacity of the embryo to produce and react to interferon (IFN) increases progressively during development; the IFN antiviral mechanism is especially strongly repressed early in embryonic life, when differentiation as well as growth are crucial processes. We have studied three quite different models of cell differentiation: (i) fibroblast-to-adipocyte; (ii) myoblast-to-muscle; and (iii) leukemic promyelocyte-to-granulocyte. In each, IFN arrests in a dose-related manner the expression of the change in genetic program. In the differentiation of the 3T3-L1 mouse embryo fibroblast, very small amounts of IFN can strikingly inhibit the cytoskeletal changes and the switches in enzymes and other proteins involved in the conversion to a fat cell. Recent studies on 3T3-L1 cells have focused on involvement of ornithine decarboxylase (ODC) and protein kinase in this activity of IFN. The inhibitory effects of IFN and an inhibitor of ODC, difluoromethyl-ornithine (DFMO) (which alone inhibits 3T3-L1 differentiation) are additive. Putrescine, the product of ODC activity, reverses the antidifferentiation activity of DFMO but does not alter the effects of IFN. These and other data suggest that ODC activity does not figure importantly in IFN action. Our recent demonstration of the entry of IFN into the cell nucleus suggests a possibly direct role of IFN for achieving its variegated effects on the genetically programmed changes during cell differentiation.

M61 THE ROLE OF PROTEIN PHOSPHORYLATION IN THE ACTIONS OF HUMAN INTERFERONS

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The phosphorylation of ribosome-associated protein P1 and the alpha subunit of protein synthesis initiation factor eIF-2 was examined in untreated and in interferon-treated human cell systems. Type I alpha IFN induced the P1/eIF-2α protein kinase in several epithelial-like cell lines including U, 293, A549, HeLa and WISH, but not in any of the human fibroblast cells that were examined including GM2767, GM52, GM54, F152 and F153. Type II gamma IFN was a poor inducer of the kinase as compared to alpha IFN. In adenovirus-infected human cells, the regulation of protein synthesis is controlled in part by a class of small virus-coded RNAs, the VA RNAs. The phosphorylation of protein P1 and eIF-2α also appears to be regulated by the VA RNAs. Ad deletion mutant dl331 which cannot synthesize the major species of VA RNA grew poorly in human cells which contain a functional P1/eIF-2α protein kinase. However, Ad dl331 grew nearly normally relative to Ad wt virus in human fibroblast cells which do not contain detectable IFN inducible P1 protein kinase. Infection of U or 293 cells with Ad dl331 result in increased phosphorylation of P1 and eIF-2α; by contrast, infection with Ad wt did not elevate the phosphorylation of either P1 or eIF-2α. Crude extracts from wt-infected cells but not from uninfected or dl331-infected cells inhibited the purified P1/eIF-2α kinase in vitro. Purified VA RNA also inhibited the purified kinase. These results suggest that phosphorylation of P1 and eIF-2α may play an important role in the response of the host to virus infection.

M62 SYNERGISM BETWEEN IFN-α AND IFN-γ IN INDUCTION OF AN IFN-INDUCIBLE mRNA, Ganes C. Sen and Jyotirmoy Kusari, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

mRNA 561 is an IFN-inducible mRNA which encodes a protein of 56kDa. We studied the characteristics of synthesis of this mRNA in HeLa monolayer cells. In response to pure IFN-αA, synthesis of this mRNA was induced rapidly. Its cytoplasmic level reached the maximum by about 6 hr and then it declined quickly even if IFN-α was present in the culture medium continuously. Little mRNA 561 was induced if an inhibitor of protein synthesis e.g. cycloheximide or anisomycin was included in the culture medium from the beginning of IFN-α-treatment. These results suggested that synthesis of an IFN-inducible protein was needed for induction of mRNA 561 by IFN-α. Pure recombinant IFN-γ could not induce the synthesis of mRNA 561 but it could apparently induce the synthesis of the putative protein that was needed for induced synthesis of mRNA 561 in response to IFN-α. Thus a high level of mRNA 561 was induced in cells that had been first treated with IFN-γ and then with IFN-α and cycloheximide. Protein synthesis was necessary either during IFN-γ-treatment or right after it to manifest this effect. The above observations are consistent with the following model of mRNA 561 induction. Either IFN-α or IFN-γ produces a signal in the cell which triggers the synthesis of a putative protein, protein X. Protein X by itself cannot induce the synthesis of mRNA 561 but it can do so in concert with a second signal which is produced by IFN-α but not by IFN-γ. Production of the second signal does not need protein synthesis. This is the first demonstration of cooperation between IFN-α and IFN-γ at the molecular level.

M63 REGULATION OF 2-5A-DEPENDENT RNase EXPRESSION

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The 2-5A-[$p_x(A2'p)_nA$, x=2 or 3, n≥2]-system is an interferon-regulated pathway which controls RNA stability in cells. Subnanomolar levels of 2-5A activate 2-5A-dependent RNase which in turn mediates some or all of the effects of the 2-5A pathway. We have reported control of cellular levels of 2-5A-dependent RNase under conditions of interferon treatment (1,2), cell growth arrest (2,3), and cell differentiation (4). In this regard, we have found that in NIH 3T3, clone 1 cells, 2-5A-dependent RNase levels were independently regulated by either interferon treatment or growth arrest (2) and in an embryonal carcinoma cell line, PC 13, the levels of the nuclease were greatly enhanced during cell differentiation (4). In general, we have found that cell lines with deficiencies in the expression of 2-5A-dependent RNase are resistant to interferon action. These results implicate 2-5A-dependent RNase in the mechanism of action of interferon. Biochemical characterization of 2-5A-dependent RNase from various mammalian sources will be compared.

(1) Jacobsen, H., Czarniecki, C.W., Krause, D., Friedman, R.M., and Silverman, R.H. (1983) Virology 125, 496-501. (2) Krause, D., Panet, A., Arad, G., Dieffenbach, C.W., and Silverman, R.H. (1985) J. Biol. Chem. 260, 9501-9507. (3) Jacobsen, H., Krause, D., Friedman, R.M., and Silverman, R.H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 4954-4958. (4) Krause, D., Silverman, R.H., Jacobsen, H., Leisy, S.A., Dieffenbach, C.W., and Friedman, R.M. (1985) Eur. J. Biochem. 146, 611-618.

Phamacology and Biological Activities

M64 INTERFERONS INHIBIT DIRECTIONAL MIGRATION OF TUMOR CELLS. G.Allavena[1], A.Melchiori[1], S.Parodi[1], L.Santi[1], J.Böhm[2], W.Remy[2], J.Schmidt[3], V.Erfle[3] and A.Albini[1]. 1-IST-Genoa(I) 2-Dermatologische Klinik T.U.-Munich(FRG); 3-GSF-Munich(FRG).

Interferons(IFNs), besides their antiviral action, are able to mediate many biological effects as inhibition of cell proliferation in vivo and in vitro and regulation of the immune response; they are currently tested as antitumoral agents in animals and in humans. Furthermore, it has been demonstrated that IFNs inhibit the motility of cultured cells. To study this last property, we have carried out experiments of chemotaxis with the Boyden chamber assay. We tested the chemotactic response of several cell lines of murine osteosarcomas induced by radionuclides, two lines of virus-transformed fibroblasts, a line of human embryonic lung fibroblasts SV40-transformed, a human fibrosarcoma and their normal counterparts (murine fibroblasts:3T3 and human embryonal fibroblasts: HEF). Different concentrations of IFNs ($2x10^3$-$2x10^4$-$2x10^5$ IRU/ml) were placed with the chemoattractant(medium conditioned by fibroblasts) for the incubation time of 8h. In the presence of the highest IFNs concentration (that was non toxic for the cells during the time of the assay) the osteosarcomas (producer of C-type particles) and the virus-transformed cells showed a reduced chemotactic activity (50-80%). Other tumoral and control lines (HEF) were completely insensitive.

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M65 ANALYSIS OF INTERFERON-MEDIATED INHIBITION OF VESICULAR STOMATITIS VIRUS REPLICATION USING CELL VARIANTS WITH DIFFERENTIAL INTERFERON SENITIVITIES. Linda S. Belkowski and Ganes C. Sen, Program in Molecular Biology and Virology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

Human and mouse cell variants differing in their sensitivities to the anti-vesicular stomatitis virus (VSV) effect of interferon (IFN) were used to investigate the basis of this effect. IFN treatment greatly reduces VSV yield in IFN-sensitive cells without any significant alteration in virus binding or internalization, suggesting that IFN may be acting directly on VSV gene expression. IFN treatment inhibits the accumulation of VSV RNA's and the synthesis of VSV proteins in IFN-sensitive cells but not in IFN-resistant cells. Using a temperature-sensitive mutant (tsG41) of VSV or wild type VSV under conditions of protein synthesis inhibition we determined that this decrease in the levels of VSV RNA's and proteins was due at least in part to an IFN-mediated inhibition of primary VSV transcription. This effect was observed only in IFN-sensitive cells and the steady-state levels of all VSV primary transcripts was reduced approximately the same extent by IFN. Northern blot analysis of primary VSV transcripts from IFN-treated sensitive cells demonstrates that the transcripts are intact but present in reduced amount. These data suggest that the inhibition of primary VSV transcription by IFN may be an important first step in the IFN-mediated inhibition of VSV replication.

M66 DIVERSITY AND EXPRESSION OF HUMAN IFN-α GENES, A.P. Bollon, Wadley Institutes of Molecular Medicine, Dallas, Texas 75235

Human IFN-α genes have been isolated from a human genomic library utilizing two 17 base oligonucleotide probes. One of the isolated genes was alpha interferon L (IFN-αL) which is a pseudogene due to a stop codon in the signal sequence coding information. Another gene that was isolated was the novel alpha interferon (IFN-αWA) which was not previously isolated from cDNA libraries. The IFN-αWA appears to represent a different group of IFN-α genes since it differs with IFN-αA-L interferons at 5 conserved amino acid positions. Two of these amino acid differences in fact involve changes from acidic to hydrophobic residues. Analysis of the IFN-αWA promoter region does not suggest any major abberations such as that found for IFN-αK which appears to be a pseudogene due to the lack of expression at the transcriptional level. The IFN-αL gene has been analyzed by correction of a translational stop codon in the signal sequence coding information. The IFN-αWA and the IFN-αL containing the corrected stop codon have been expressed in E. coli using a LacZ expression system. Hence the IFN-αWA and the IFN-αL mature coding sequences do in fact code for active interferons. These results bear on the diversity of the IFN-α gene family as will be discussed. Expression of the IFN-αWA and IFN-αL genes, analysis of the gene products, and the presence of potential procarytoic translational control sites which could generate truncated IFN-α in bacteria will be discussed. The interaction of these alpha interferons with genetically engineered human tumor necrosis factor (TNF) is also under analysis with emphasis on a comparison of IFN-αWA and IFN-αD, which is one of the standard alpha interferons.

M67 EFFECTS OF INTERFERON ON THE REPAIR OF RADIATION INDUCED POTENTIALLY LETHAL AND SUB-LETHAL DAMAGE IN HUMAN TUMOR CELLS. Alex C.Y. Chang, Peter C.Keng, University of Rochester Cancer Center, Rochester, New York 14642

It has been previously reported by us that radiation cytotoxicity in human tumors was enhanced by treatment with interferon(IFN) at 24 hr prior to irradiation. The enhancement was shown to be time dependent and cell cycle specific. This study was designed to determine whether the mechanism of interaction between interferon and radiation was related to the cellular repair capacity of human tumor cells.

Human hypernephroma ACHN and squamous carcinoma A431 cells were grown in monolayer culture in MEM medium supplemented with 10%(ACHN) or 20%(A431) fetal calf serum. A single dose of 1000 IU/ml rHuIFN-r (Hoffman LaRoche) was given to fed plateau and exponentially growing cultures for 24 hr prior to the studies of potentially lethal damage repair(PLDR) and sublethal damage repair(SLDR). A single dose of 12 Gy or two doses of 6 Gy gamma-ray was used to induce PLD and SLD. Flow cytometry(FCM) analysis was also employed to monitor the cell cycle redistribution during the repair processes.

Preliminary data showed that both ACHN and A431 cells expressed PLDR with a 10-20 fold increase in cell survival during the first 12 hr after irradiation. However this repair capacity was reduced to 5-10 fold by pretreatment with IFN, suggesting IFN inhibited PLDR. DNA histograms measured by FCM did not show significant difference in cell cycle redistribution during PLDR between radiation only and IFN plus radiation treatment. Work is in progress to use fractionated radiation dose to determine the effect of IFN on SLDR. This work was supported in part by NIH Grants CA-11051 and CA-11198.

M68 **EFFECT OF INTERFERON ON RIBONUCLEOTIDE AND DEOXYRIBOUCLEOTIDE METABOLISM IN HUMAN LYMPHOBLASTOID CELLS, Amos Cohen, Jerzy Barankiewicz and Chaim Kaplinsky, The Hospital for Sick Children, Research Institute, Division of Immunology, 555 university Avenue, Toronto, Ontario, Canada M5G 1X8**

The effect of recombinant α_2-interferon (50 units/ml), on the cell cycle, nucleotide metabolism, protein and nucleic acid synthesis was studied in human B-lymphobastoid (Daudi) cells. Cell cycle analysis showed that interferon treatment resulted in G_o/G_1 arrest (68%) as compared to control cells (40% at G_o/G_1). Interferon inhibited the incorporation of radioactive thymidine and uridine into DNA and RNA respectively, but had little effect on the incorporation of amino acids into proteins. Interferon inhibited the biosynthesis of ribonucleotides by both de novo and salvage pathways and decreased the level of phosphoribosylpyrophosphate, the rate limiting substrate for purine and pyrimidine nucleotide biosynthetic pathways. Interferon also markedly ihibited deoxyribonucleotide synthesis by both ribonucleotide reuction and deoxyribonucleoside salvage. In contrast, ribonucleotide catabolism was significantly increased in the presence of interferon while no changes in ribonucleotide interconversion were observed. The substantial increase in ribonucleotide and deoxyribonucleotide biosynthetic rates combined with the increase in ribonucleotide degradation rate results in decreased availability of intracellular nucleotides and deoxyribonucleotide pools.

These results suggest that inhibition of ribonucleotide and deoxyribonucleotide synthesis together with increased rate of nucleotide catabolism may diminish the availability of nucleotides for nucleic acid synthesis thereby causing cell growth arrest.

M69 Differential Sensitivity of B-16 Melanoma Cells to Prolonged Treatment with Murine α, β, and γ Interferons. W.R. Fleischmann, Jr. and C.M. Fleischmann, The University of Texas Medical Branch, Galveston, TX 77550

Interferons have potent antiproliferative activities. To investigate the mechanisms by which interferons exert their antiproliferative activities, mouse B-16 melanoma cells were cloned with the different murine interferons for various initial periods of time (1/2, 1, 2, 4, or 6 days), washed to remove residual interferon, overlaid with fresh growth medium, and reincubated for the remainder of an 8 day cloning period. Untreated cells and cells treated with interferons for 8 days were employed as negative and positive controls. IFN-α and IFN-β treatments had similar effects. Maximal inhibition of clone number was attained with 2 to 4 days of interferon treatment. Moreover, the number of cells per clone was the same, whether cells were treated for 2 days or for 8 days with interferon. These results suggest that B-16 melanoma cells were initially sensitive to IFN-α and IFN-β but became resistant after 2 to 4 days of treatment. In contrast, cells produced progressively fewer clones when treated with IFN-γ for progressively longer periods of time. Also, the number of cells per clone diminished with increasing time of interferon treatment. Thus, the cells did not become resistant to IFN-γ. Combination treatment with IFN-γ plus IFN-α or IFN-β potentiated the antiproliferative effect and mirrored the IFN-γ effect, except that the curve showing the progressive sensitivity of the cells was markedly more steep with the combination treatment. These results may have clinical ramifications since they suggest that IFN-γ may have more antitumor potency than IFN-α or IFN-β and that combination interferon therapy may be the most potent.

M70 POTENTIATED ANTITUMOR EFFECTS OF INTERFERON-α AND MISMATCHED DOUBLE-STRANDED RNA (AMPLIGEN) IN VITRO AND IN VIVO, H.R. Hubbell, E.C. Pequignot, K.R. Shanabrook, W.A. Carter, R.D. Williams, and D.R. Strayer, Hematology/Oncology, Hahnemann University, Philadelphia, PA, and Urology, University of Iowa, Iowa City, IA.

The antitumor effects of human IFN-α and Ampligen, alone and in combination, against a human renal cell carcinoma cell line, 786-0, were studied both in a clonogenic soft agar assay and in the nude mouse. Synergistic antitumor effects were seen in vitro, even though IFN-α alone was not effective over the range of concentrations studied. Ampligen alone (500 μg 3 times per week IP) and in combination with IFN-α (20,000 IRU daily IP) were both effective ($p<0.001$) against the nude mouse xenografts with significant increase in survival ($p<0.001$ for both groups). IFN-α alone (20,000 IRU daily IP) did not inhibit tumor growth, however, significant titers (>3,000 IRU/ml) were found in the serum shortly after treatment. Assays of mouse IFN induction and in vitro antigrowth effects indicated that the in vivo antiproliferative effect of Ampligen was probably not due to potentiation of the direct effects by the induced IFNs. The tumor growth inhibition appeared to occur from the significant augmentation ($p<0.01$, compared to controls) of natural killer cell activity in spleen cells by Ampligen. Combined IFN-α/Ampligen treatment further augmented NK cell activity ($p<0.05$, compared to all other treatments tested) by rendering the target cells more sensitive to lysis, even though IFN-α alone could not activate NK cells, nor increase target cell sensitivity. These results indicate that Ampligen and IFN-α can act together to potentiate both direct antitumor and indirect immunomodulatory effects.

M71 QUANTITATION OF MISMATCHED DOUBLE-STRANDED RNA IN PLASMA USING NUCLEIC ACID HYBRIDIZATION L.J. Krueger, D.R. Strayer, P.J. Andryuk and M.J. Borigini, Department of Hematology/Oncology, Hahnemann University, Philadelphia, PA 19102

Mismatched double-stranded RNA, poly(I)·poly(C_{12},U) induces measurable quantities of the interferons, as well as activates the mediators of the interferon response. Studies of this drug in tissue culture systems, animal models, and clinical trials have demonstrated antitumor effects. The mechanism of this anticellular effect remains unknown. We have developed a new method for the measurement of dsRNAs in biological fluids. Quantifying dsRNA should not only be useful in measuring and identifying patient parameters which would facilitate the utilization of the therapeutic potential of the dsRNAs, but should aid in the understanding of the structural requirements of dsRNA antineoplastic action. Protein concentration, digestion and denaturation temperatures were shown to have profound effects on the linearity and extent of hybridization. This method is sensitive to a clinically attainable concentration ($10\text{-}100\times10^{-9}$) and can be used in pharmacokinetic studies of treated patients. Conditions for the quantitation of drug levels in plasma were optimized. Results indicate marked variation in patient dsRNA levels (half-lives varied 0-53 minutes). Future studies will correlate plasma levels with immunological and antitumor effects in patients receiving this antineoplastic agent.

M72 EFFECT OF INTERFERONS ON MURINE LYMPHOCYTE RECIRCULATION. Eric A. Mann and Donna M. Murasko, The Medical College of Pennsylvania, Philadelphia, PA 19129

We previously demonstrated a transient decrease in the number of thoracic duct lymphocytes(TDL) in mice injected with exogenous interferon(IFN) or IFN-inducers. Although a similar decrease in TDL may occur in humans, only decreases in peripheral blood leukocytes(PBL) have been reported with IFN therapy. Our present studies show that IFN and poly I:C, at less than half the dose required to decrease the TDL, decrease the number of PBL. This effect appears 6h following IV injection of IFN, peaks at 24h, and disappears by 48h. Although IFN-α is more effective than IFN-β in causing initial decreases in PBL, similar maximum decreases were seen at 24h. Analysis of the cells shows that all populations are equally decreased in TDL. In contrast, the decrease in leukocytes in PBL is due to a decrease in lymphocytes; the number of neutrophils is increased. To determine the mechanism of these decreases, TDL were labelled with ^{51}Cr, injected into syngeneic mice at various times before and after IFN treatment and migrational patterns were determined. In mice injected with poly I:C 12h after inoculation of labelled TDL, small, but reproducible, decreases in lung and liver and reciprocal increases in spleen and lymph nodes were noted 1 and 4h later. These results were obtained by radioactive counts of the organs and autoradiography of tissue sections. Treatment with poly I:C 6h prior to inoculation of TDL resulted in a 50% increase in lung and liver with a reciprocal decrease in spleen and lymph nodes at 1 and 4 h. We postulate that IFN exerts its effects on host tissue, rather than on lymphocytes, since in vitro treatment of TDL with IFN for 6h causes no alteration in migration patterns. Future studies will examine the effect of this decrease on the generation of immune responses. (Supported by AI 16840)

M73 SYNERGISM BETWEEN IFNγ AND TUMOR NECROSIS FACTOR IN THE INDUCTION/ENHANCEMENT OF HLA EXPRESSION IN LEUKEMIC AND CARCINOMA CELL LINES, Klaus Pfizenmaier, Martin Krönke and Peter Scheurich, Clinical Research Group BRWTI, Max-Planck-Society, 3400 Göttingen, F.R.G.

There is ample evidence that IFNγ enhances HLA class I and class II antigen expression and affects growth and differentiation of both malignant and nonmalignant cells from various tissue origins. With respect to its antitumoral activity, the observation of strong synergistic action together with Tumor Necrosis Factor is of particular interest. To date, these observations have been confined to the growth inhibitory/cytotoxic action of the two cytokines. We have investigated, whether TNF and IFNγ might have a broader spectrum of synergistic activities and analyzed the modulation of HLA expression in leukemic and colon carcinoma cell lines. The data obtained provide evidence that TNF, which on its own has no or only a moderate influence on HLA expression, can strongly enhance the IFNγ induced expression of HLA-DR antigens on cell lines sensitive to this IFN effect. No induction of HLA-DR was achieved in cell lines which were in this respect resistant to IFNγ alone. However, in HLA-DR non-inducible cells combined treatment with TNF/IFNγ resulted in strongly enhanced expression of HLA ABC antigens. We are currently investigating whether TNF enhances the IFNγ induced HLA gene expression at the transcriptional or a post-transcriptional level.

M74 THE AUTOCRINE SYSTEM FOR IFNα/β AND TGFβ IN THE MOUSE C-243 CELLS : THE DOMINANCE OF TGFβ, Waldemar Popik and Anna D. Inglot, Institute of Immunology and Experimental Therapy, 53-114 Wrocław, Poland

The clone of mouse C-243 cells adapted to growth in suspension was found to produce both IFNα/β and a growth factor which has all properties of TGFβ. TGFβ from C-243 cells was purified to homogeneity. C-243 cells are sensitive to both IFNα/β and TGFβ. TGFβ acts as antagonist of IFN. C-243 cells grown as large subcutaneous tumors in irradiated or non-irradiated mice can be stimulated for increased proliferation by treatment either TGFβ or with antibodies neutralizing IFNα/β. On the other hand IFNα/β inhibits the growth of C-243 tumors. The progresive growth of C-243 tumors and the transformed morphology of the cells appear to be due to TGFβ. Although TGFβ is a conditional inhibitor of some cells in our system it appears to act only as a growth stimulant in contrast to IFNα/β which function as growth inhibitors.

M75 EVALUATING THE ENHANCEMENT OF TUMOR NECROSIS FACTOR CYTOTOXICITY, James C. Salwitz, Robert B. Dickson, Marc E. Lippman, NCI, Bethesda MD.

Tumor Necrosis Factor(TNF) is a cytotoxic lymphokine which has been evaluated in a number of short term tumor cell toxicity assays. In this study a comparison was made of the effects of TNF in long term cell cloning assays, such as monolayer and soft agar cloning, and short term cell killing assays, such as 18 hour cell killing and 48 hour thymidine label assays. It was found that long term assays reproduced the results seen in short term assays, but were more sensitive to differences in cell line susceptibility to TNF inhibition. Interferon synergy with TNF could be easily demonstrated and quantitated. T47D human breast cancer cells, which were relatively resistent to TNF, were quite sensitive to TNF in the presence of a non-toxic dose of gamma interferon. These techniques were used to evaluate previously unreported synergistic agents, such as lupeptin and monensin. Long term cloning assays offer reliable, accurate and simple methods of evaluating TNF activity, and potential synergistic agents.

M76 INHIBITION OF HERPES SIMPLEX VIRUS TYPE 1 mRNA ACCUMULATION BY INTERFERON, Opendra K. Sharma, Biswendu B. Goswami, and Randall J. Cohrs, AMC Cancer Research Center, Denver, Colorado 80214

Human recombinant interferon α, β, γ or natural interferon β effectively inhibited replication of HSV-1 in Vero cells. Interferon reduced the steady state levels of HSV-1 mRNAs for ICP-4 and ICP-0 (immediate-early proteins), followed by inhibition of synthesis of the early proteins, Tk (thymidine kinase), ICP-8 (DNA binding protein) and early-late protein (major capsid protein, ICP-5). A corresponding reduction in the steady state levels of mRNAs for Tk and ICP-5 was also observed. Interferon inhibited ICP-5 mRNA accumulation completely and reduced ICP-4 mRNA level by 80-90% at 1 h and 3 h post-infection, respectively. However, there was a marked difference in response to interferon treatment on the accumulation of ICP-0 and Tk mRNAs in cytoplasm. Initially accumulation of both of these mRNAs was completely inhibited by interferon, however, with an increase in time after infection, increased amounts of both mRNAs were detected in cytoplasm. By 5 h post-infection ICP-0 and Tk mRNAs were present at 43% and 31% respectively of levels of these mRNAs in untreated cells. The effect of interferon and virus infection on the steady state levels of β-actin mRNA and integrity of 28S rRNA was determined to ascertain whether the reduction of virus specific mRNAs was due to their degradation. No significant reduction in the cytoplasmic levels of β-actin mRNA was observed in interferon treated virus infected cells, compared to untreated infected cells. Furthermore, the degradation of rRNA into characteristic pattern of product which is an indicator of 2-5A dependent endonuclease activation was not seen. The res presented here suggest a differential effect of interferon on HSV gene expression.

M77 MECHANISM OF THE SYNERGISTIC ANTIPROLIFERATIVE EFFECT OF INTERFERON ALPHA 2 WITH BLEOMYCIN ON A HUMAN LEUKEMIA CELL LINE (K 562). L.Silvestro,I.Viano,G.Compagnoni and E.Genazzani,Institute of Pharmacology,Medical School,University of Turin - Italy

In previous experiments we found a synergistic antiproliferative effect of interferon alpha 2 (IFN) with bleomycin (BLM) on K 562 cell line (I.Viano, L.Silvestro et al.,Abstracts book 1985 TNO-ISIR meeting on the interferon system).BLM and IFN alone are quite ineffective against this cell line; we have evaluated the IFN effect on growth kinetic,BLM uptake and cellular fate of this drug as possible mechanism of this sinergy.Cell kinetic,measured with labeling index, with the low doses of IFN used is slightly affected. Intracellular levels of BLM,evaluated with a biological assay against B.Subtilis,do not change after IFN treatment.Bleomycinase activity,tested with the same biological assay , in homogenate of cells receiving IFN is unchanged .The inibithion of DNA repair mechanisms or an enhancement of the DNA damage induced by bleomycin seem to be promising hypothesis to explain this synergy.Now we are evaluating these suggestions and the results will be shown

M78 SYNERGISTIC ANTITUMOR EFFECT OF INTERFERON-α AND MISMATCHED DOUBLE-STRANDED RNA AGAINST FRESH HUMAN TUMOR CELLS, David R. Strayer, Pauline Watson, Sheila Mayberry, and William A. Carter, Department of Hematology/Oncology, Hahnemann University, Philadelphia, PA 19102

The antitumor activity of human leukocyte interferon-α (IFN-α) obtained from the New York Blood Center and mismatched double-stranded RNA (Ampligen) were studied individually and in combination on fresh human solid tumor biopsy samples using a clonogenic assay. Single cell suspensions of tumor cells were plated in triplicate in soft agar in the presence or absence of IFN-α, Ampligen, or both, and colonies (> 40 cells) were counted at 15-20 days. Adequate colony formation to evaluate IFN-α/Ampligen sensitivity (>30 colonies/plate) was obtained in 53 cases. A synergistic antitumor effect of the combined IFN-α (1000 units/ml) and Ampligen (250 μg/ml) treatment was seen in 2/6 breast carcinomas (33%), 2/5 ovarian carcinomas (40%), 4/8 melanomas (50%), and 20/34 renal cell carcinomas (59%). The antiproliferative effect of IFN-α against renal cell carcinoma was potentiated > 20-fold (mean) by the synergistic interaction with Ampligen. While the sensitivity (>60% decrease in colony formation) of renal tumors to IFN-α (24%) and Ampligen (21%) individually was low, 22/34 samples were sensitive to the combinational treatment. Although clinical trials coupled with in vitro clonogenic studies will be needed to better define the therapeutic potential of this combination, these results indicate that IFN-α and Ampligen in combination may be more effective at exerting an antiproliferative effect than either agent alone.

M79 PROPERTIES OF MODIFIED MOUSE INTERFERON ALPHA SPECIES, Jan Trapman, Margreet van Heuvel, Ellen Zwarthoff, Jaap Bosveld and Paul Klaassen, Department of Pathology, Erasmus University, Rotterdam, The Netherlands

Five different mouse interferon alpha (Mu IFN-α) genes (α1,2,4,6 and 9) were isolated and the amino acid sequence as deduced from the nucleotide sequence determined. All five genes code for a biologically active protein. Four out of five proteins contain a putative N-glycosylation site at amino acid position 78 to 80. Cysteine residues are found in all five proteins at positions 1, 29, 86, 99 and 139. Mu IFN-α4 has a deletion of five amino acids at position 103 to 107. If transiently expressed in COS cells most antiviral activity is produced by this α4 gene. α1 and α6 show the highest activity on heterologous cells.

Hybrid genes were prepared between the α1 and α4 and the α2 and α4 gene using common restriction enzyme recognition sites. α2/α4 hybrids produce, in contrast to α1/α4, α4/α1 and α4 /α2 hybrids large amounts of antiviral activity. Site-directed mutagenesis experiments show that α1 and α2 modified in the N-glycosylation site have the same antiviral activity as the original proteins. Moreover, it was found that cysteine-99 is not essential for biological activity. Further experiments on structure/function analysis of Mu IFN-α proteins are in progress.

M80 CHARACTERIZATION AND CLONING OF AN INTERFERON REGULATED dsRNA BINDING PROTEIN USING A SPECIFIC MONOCLONAL ANTIBODY, Linda J.Z. Penn, Hunt Willard, and Bryan R.G. Williams, Hospital for Sick Children and Departments of Microbiology and Medical Genetics, University of Toronto, Toronto, Ontario, M5G 1X8.

A monoclonal antibody (MAb) (10A5) has been previously described which demonstrates specificity to a dsRNA-binding phosphoprotein of approximate 68K Mr which is localized to the cell cytoplasm and elevated 2-3 fold with interferon (IFN) treatment[1]. Although these characteristics of the 10A5 antigen ressemble those of IFN induced, dsRNA binding protein kinase, analysis of mouse/human hybrid cells by immunoblotting and human protein kinase activity suggest the two proteins are not identical. Interestingly, immunoblotting the hybrid lysates with MAb 10A5 revealed the 10A5 antigen is localized to human chromosome 12, where the genes of two other IFN induced proteins have also recently been localized[2]. To further characterize the 10A5 antigen, a human cDNA expression lambda gt11 library was screened with MAb 10A5. A cDNA clone was isolated which encodes a fusion protein binding to MAb 10A5 in immunoblotting. Northern blot analysis showed this cDNA binds to a single RNA transcript (3.2kb) in human but not mouse cells.

1. Penn & Williams, 1985 PNAS 82:4959.
2. Saunders et al, 1985 In, The 2-5A System: Clinical and Molecular Aspects of the Interferon Regulated Pathway (R.Silverman & B.Williams, eds) Alan R. Liss, NY (In press)

M81 INDUCTION OF INTERFERON-BETA BY A FIBROBLAST GROWTH FACTOR ("TUMOR NECROSIS FACTOR"): A HOMEOSTATIC MECHANISM IN THE CONTROL OF CELL PROLIFERATION.
Masayoshi Kohase, Dorothy Henriksen-DeStefano and Jan Vilček, New York University Medical Center, New York, N.Y. 10016.
Earlier studies showed that tumor necrosis factor (TNF) exerts a potent mitogenic effect in human diploid fibroblasts. Here we demonstrate that highly purified E. coli-derived recombinant human TNF induces an antiviral state against encephalomyocarditis virus in human FS-4 foreskin fibroblasts. Induction of the antiviral state by TNF was demonstrable in confluent, "aged" FS-4 cells but not in freshly seeded cultures. Antibodies to human IFN-alpha or IFN-gamma did not prevent development of the antiviral state induced by TNF. However, addition of neutralizing antibodies specific for human interferon (IFN)-beta inhibited development of the TNF-induced antiviral state, indicating that it is mediated by the generation of IFN-beta. Furthermore, a concentrate of culture fluid from FS-4 cells exposed to TNF was shown to contain small quantities of antiviral activity that was abolished in the presence of antiserum to human IFN-beta. We also show that antiserum to human IFN-beta enhanced the mitogenic effect of TNF in confluent serum-starved FS-4 cells. Since IFN-beta inhibits the mitogenic action of TNF, induction of IFN-beta by TNF and some other growth factors (e.g., interleukin 1 or platelet-derived growth factor) may represent a physiological negative feedback mechanism regulating cell proliferation. (Supported in part by NIH grant AI-12948 and by a grant from Suntory Limited.)

M82 DIFFERENTIAL EFFECTS OF INTERFERONS ON THE BINDING OF TUMOR NECROSIS FACTOR TO RECEPTORS IN TWO HUMAN CELL LINES.
Masafumi Tsujimoto, Rena Feinman and Jan Vilček, New York University Medical Center, New York, N.Y. 10016.
In earlier studies we showed that IFN-gamma increased tumor necrosis factor (TNF) binding in several transformed cell lines and in human foreskin fibroblasts. Enhanced TNF binding was shown to be due to an increased synthesis and expression of TNF receptors in cells incubated with IFN-gamma (Tsujimoto et al., in press). In the present study we examined the effect of IFN-alpha and IFN-beta on the binding of ^{125}I-TNF to receptors in HeLa and HT-29 cells. In HeLa cells both IFN-alpha or -beta increased TNF binding, whereas in HT-29 cells these two IFNs slightly decreased TNF binding. Furthermore, IFN-alpha and -beta exerted an antagonistic effect on IFN-gamma-induced stimulation of TNF binding in HT-29 cells. This finding is reminiscent of the antagonistic effect of IFN-alpha and -beta on IFN-gamma-induced expression of Ia antigen in murine monocytes (Ling et al., J. Immunol. 135, 1857, 1985). Despite the inhibitory effect on TNF binding, IFN-beta and TNF acted synergistically in producing an antiproliferative effect in HT-29 cells, both in the presence and absence of IFN-gamma. Thus, synergism between IFNs and TNF can occur by mechanisms separate from the effects of IFNs on TNF receptor expression. (Supported in part by NIH grant AI-12948 and by a grant from Suntory Limited.)

M83 MECHANISMS OF ACTION OF INTERFERONS IN FRIEND CELLS: ANTIVIRAL STATE AND DIFFERENTIATION, Giovanni B. Rossi[1,2], Maurizio Federico[1], Eliana M. Coccia[1], Elisabetta Affabris[2], Giovanna Romeo[1], Departments of [1]Virology, Istituto Superiore di Sanità, Rome and [2]Cellular and Developmental Biology, University "La Sapienza", Rome, Italy.

Friend leukemia cells (FLC) are mouse nucleated erythroid precursors able to differentiate in vitro when treated with dimethylsulfoxide (DMSO). Variant sublines of FLC resistant to the α,β interferon (IFN)-induced antiviral state (1) have been isolated in our laboratory. Both IFN-resistant and wild-type FLC show a specific saturable binding site for ^{125}I-mouse α,β pure IFN. Cell extracts of α,β IFN-treated variants do not show any induction of the IFN-induced dsRNA-dependent 2-5A synthetase (2) and the 67K protein kinase activities. On the contrary, these variants are susceptible to γ IFN: yet no inducible 2-5A synthetase activity is detectable in extracts of cells exposed to γ IFN. The 67K protein kinase activity is, instead, induced. It appears therefore that FLC variants can develop a bona fide antiviral state in the absence of any inducible 2-5A synthetase activity (3).

The responses of our α,β IFN-resistant FLC clones to α,β or γ IFNs with respect to the modulation of erythroid differentiation are summarized herebelow:

	modulation of differentiation by α,β IFN		modulation of differentiation by γ IFN
cell clones	low doses	high doses	
wild type	enhanced	inhibited	inhibited
resistant	none	inhibited	inhibited

Analysis of induction of 2-5A synthetase and 67K protein kinase activities, when resistant FLC clones are induced to differentiate and treated with IFNs, suggests that these enzymatic pathways are not involved in the inhibition of erythroid differentiation mediated by IFNs. With respect to the γ IFN treatment of resistant clones, only a 4-day exposure to DMSO and natural or recombinant γ IFN results in the detection of a small but significant 2-5A synthetase activity undetectable. Studies of 2-5A synthetase mRNA expression show no induction of 2-5A synthetase mRNA in these variants when the cells are treated with 200 U/ml of α,β IFN and analyzed by Northern blot using a cDNA probe for human 2-5A synthetase.

1) Affabris et al., Virology 120: 441, 1982.
2) Affabris et al., Virology 125: 508, 1983.
3) Romeo et al., J. Biol. Chem. 260: 3833, 1985.

M84 INTERFERON (IFN)-DEPENDENT-REGULATION OF VIRUS-INFECTED TARGET CELL SUSCEPTIBILITY TO NATURAL KILLER (NK) CELLS AND CYTOTOXIC T LYMPHOCYTES (CTL), Raymond M. Welsh and Jack F. Bukowski, Univ. Mass. Medical School, Worcester, MA 01605.

A variety of experimental approaches have indicated that NK cells mediate natural resistance of mice to murine cytomegalovirus (MCMV) but not to lymphocytic choriomeningitis virus (LCMV). Class I histocompatibility antigen (H-2)-restricted CTL regulate the LCMV infection. Both virus infections induce IFN, which activates NK cells and simultaneously induces a state of resistance to NK cell-mediated lysis in target cells. Bone marrow cells and thymocytes isolated from IFN-treated or LCMV-infected mice have markedly increased H-2 antigen expression and enhanced susceptibility to allospecific CTL-mediated lysis but are resistant to NK cells. LCMV-infected target cells treated with IFN become resistant to NK cells but are much more sensitive to virus-specific CTL. In contrast, MCMV-infected target cells treated with IFN remain susceptible to NK cells. These diverse IFN-mediated effects on virus-infected target cells may account for the relative roles of NK cells and CTL in regulating different virus infections.