MINIREVIEW

Interferons and Interferon Inhibitory Activity in Disease and Therapy

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Interferon (IFN) resistance is an important factor in the pathophysiology of neoplastic disorders, certain viral infections (e.g., AIDS), and autoimmune diseases (e.g., lupus erythematosus and Wegner's granulomatosis). In addition, in some of these disorders, there is also decreased ability to produce IFNs. The capacity of viruses and neoplastic processes to interfere with the IFN system are thought to represent a "virus-against-host" or "cancer-against-host" defense mechanism. Four resistance factors have been identified: 1) release of free IFN-α/β type 1 receptors into the circulation that, at appropriate concentrations, capture and inactivate IFNs; 2) a new IFN inhibitory protein has been isolated and its chemical structure is under study; 3) prostaglandin E_2, which is produced by certain tumor cells, inhibits IFN production; and 4) high levels of cAMP phosphodiesterases present, for example in certain tumor cells, reduces cAMP, an important second messenger in IFN synthesis. Studies are under way to reverse these inhibitory effects and to increase endogenous interferon production. Exp Biol Med 229:285–290, 2004

Key words: interferon inhibitors; α/β type 1 interferon receptors; pathophysiology of neoplastic diseases; viral infections; AIDS; autoimmune diseases

Interferon (IFN) Production and Its Modifications

IFNs are a group of naturally occurring proteins and glycoproteins known to have antiviral, antiproliferative, and immunoregulatory effects. Normal healthy individuals do not have demonstrable levels of IFN in their circulation. IFNs appear in blood in response to infection, at clinical and subclinical levels, or in response to antigen and mitogen simulation. The appearance of IFN in the circulation constitutes an initial line of host defense against infection. Normal healthy individuals can be broadly classified into two categories on the basis of the ability of their lymphocytes to synthesize IFN-α in response to a viral or nonviral challenge: "average producers" (>2000 IU/ml) and "low producers" (<1000 IU/ml; Ref. 1). It is conceivable that low producers of IFN are more prone to infection than normal producers. The question has arisen as to whether they are also more likely to develop neoplastic disorders. Studies have been performed to investigate IFN synthesis ability of white blood cells of patients with various disorders in comparison to normal individuals. A significant decrease in IFN synthesis ability was observed in patients with AIDS and with cancer (2, 3).

Immunosuppressant agents (cyclosporin and prednisone), which are used in organ-transplant recipients and in the therapy of autoimmune disease, suppress IFN production (4). Prostaglandin E_2 (PgE_2) and cAMP-reducing factors also inhibit IFN synthesis. Although PgE_2 increases cAMP production, high levels of cAMP-phosphodiesterases seem to offset this effect. On the other hand, PgE_2 inhibitors (e.g., indomethacin and meclofenamic acid) and cAMP-increasing phosphodiesterase inhibitors (e.g., pyrimidopyrimidine and methylxanthine derivatives) increase IFN-α synthesis and convert "low-producer" white cells into "normal-producer" white cells *in vitro* (1, 4–6). In tumor cells and in the circulation of patients with cancer, high levels of PgE_2 have been found; this was considered to be one of the tumor-against-host defense mechanisms. Neoplastic cells also contained high cAMP-phosphodiesterase

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levels. These factors appeared to be involved in interference with IFN synthesis (5–7).

Meclofenamic acid, a double inhibitor of both the cyclooxygenase and lipoxygenase pathways of the arachidonic acid metabolism and, hence, PgE_2 production (8), has been shown to increase IFN-α production in human lymphocytes without interfering with the antiviral and antiproliferative effect of IFN-α (9). In a murine model of AIDS and related lymphomas (LP-BM5-MULV virus infection of C57/Bl/6 mice), decreased IFN production appeared to play a role in the pathophysiology of the disease. Treatment with meclofenamic acid and pentoxifyline (a cAMP-phosphodiesterase inhibitor and cAMP-increasing agent) increased IFN production and decreased biochemical and hematologic manifestation of the disease, including the L_3T_4:Lyt2 ratio (corresponding in mice to the human T_4:T_8 cell ratio), splenomegaly, and lymphadenopathy (10). Because animals were killed at various times after infection in these studies, no survival data have been reported.

IFN-α was found to alter membrane potentials in IFN-sensitive Daudi cells (from patients with Burkitt's lymphoma). IFN produced dose-dependent hyperpolarization, decreased membrane viscosity, and modulation of the microfilament system. These effects were not altered by meclofenamic acid or the cAMP-phosphodiesterase inhibitor pentoxifylline (11). Another cAMP-phosphodiesterase inhibitor, the pyrimido-pyrimidine derivative mopidamole was found to potentiate the neoplastic growth inhibitory effect of IFN-β in several human cancer cell lines in tissue culture (12).

Resistance to IFN Therapy and Role in Pathophysiology

It is well recognized that IFNs can be used for the treatment of certain viral infections and certain neoplastic disorders, including hematologic malignancies and AIDS-related neoplastic complications (13, 14). IFNs have been used alone or in combination with many other forms of treatment. Among the vast majority of clinical trials done thus far, IFNs induce remission in some patients, whereas, in the great majority of clinical trials, they have at best led only to minor improvements. In the most commonly occurring solid cancers such as cancer of the lung, breast, colon, and prostate, IFNs have been largely ineffective (13–17). The reasons for the lack of response *in vivo* are not fully understood. Available data suggest that the lack of response is partly due to the appearance of IFN inhibitory activity present in the circulation of these patients (18–23). Modest inhibitory activity was also found in normal body fluids, nasal secretions, and urine (24–26). These inhibitors appeared to be independent of antibodies produced during IFN therapy (27).

In a group of patients with hemophilia who were infected with human immunodeficiency virus–contaminated blood preparations at a known time, IFN levels were measured. They increased after infection and were mostly of the acid-labile α type (most IFN-α types are acid stable). This type of IFN was thought to be diagnostic for AIDS, but it is also seen in lupus erythematosus (SLE). After a variable latent period, patients developed clinical AIDS. At that time, IFN inhibitory activity became measurable in the circulation (13, 18, 22, 23, 28). It was thought that the induction of IFN inhibitor production is a "virus-against-host" defense mechanism.

A search of IFN inhibitory activity in a wide variety of clinical conditions revealed the presence of such activity in certain autoimmune disorders, including SLE with vasculitis (28–30) and Wegner's granulomatosis (31). Some of the highest IFN inhibitory activity was found in patients who developed AIDS-related lymphomas and in patients with AIDS-related Kaposi sarcoma (3, 6, 18, 22, 23). A further search in neoplastic diseases revealed high levels in the majority of all patients with cancer. When the neoplasms were successfully removed surgically or were significantly reduced by radiation and/or chemotherapy, there was elimination or decrease of IFN inhibitory activity, which suggests that at least some of the inhibitory activity was produced by neoplastic cells (3, 6, 23, 32).

In the studies referred to above, overall inhibitory activity was measured by mixing a series of dilutions of patients' serum/or purified IFN inhibitory preparation with 100 IU of natural human IFN-α (nHuIFN-α), incubated at 37°C for 2 hrs, and then assayed for the remaining IFN antiviral activity according to the method of Finter (33). The following controls were used in all IFN inhibitor assays: 1) patients' serum or inhibitor preparation mixed in equal proportions with culture medium to determine any carryover IFN activity, 2) an appropriate dilution of pooled normal human serum mixed with 100 IU of nHuIFN-α to document that no IFN inhibitory activity was present in normal serum, or 3) 100 IU of nHuIFN-α mixed with culture medium. All controls were treated identically to IFN inhibitor preparations. One unit of IFN inhibitory activity was defined as the amount that can block antiviral activity of 25 IU of nHuIFN-α. The inhibitory titer was expressed as the reciprocal of the dilution of serum needed for 1 U of activity. IFN inhibitory activity is not very stable when serum is kept frozen at –70°C; nearly 90% of its activity is lost after 6 months of storage at this temperature. For some studies, serum containing IFN inhibitory activity was precipitated with ammonium sulfate. The precipitate, at 50% ammonium sulfate concentration, contained all inhibitory activity. It was dissolved in 20 m*M* sodium phosphate buffer (pH 7.0), dialyzed against the same buffer, chromatographed, and lyophilized in small aliquots. This resulted in the preservation of IFN inhibitory activity for a minimum of 1 year.

The relationship of IFN activity and production to temperature has been studied (34). Mild hyperthermia (39°C) increased IFN-α, -β, and -γ antiviral and antiproliferative activity *in vitro* in human and murine normal and neoplastic cell cultures but decreased IFN production on stimulation

by Sendai virus or staphylococcal endotoxin. Mild hyperthermia decreased the IFN-induced enhancement of natural killer cell (NKC) activity (34).

Of all the clinical conditions studied, the highest levels of IFN inhibitory activity were found in patients with AIDS, particularly if it was complicated by neoplastic diseases (e.g., B cell lymphomas or Kaposi sarcoma; Refs. 3, 18, 22, 23, 28, 35), followed by patients with neoplastic disorders (3, 6, 23, 32) and autoimmune disease (SLE or Wegner's granulomatosis; Refs. 28–30, 35). The mechanism of action of this inhibition was studied in more detail, and several mechanisms were uncovered (28, 30).

It appeared that, in the circulation, free IFN-α/β type 1 receptors appeared in concentrations that capture and inhibit the activity of IFN-α and -β. Hardy *et al.* (36) reported that, in mice, free IFN receptors can be both agonists and antagonists, depending on the concentration. The levels reported in patients were of the antagonist level. This was also born out by clinical and laboratory-proven resistance to IFN therapy. Moosmayer *et al.* (37) and Ozmen *et al.* (38) showed that the free IFN-γ receptor is an effective inhibitor of IFN-γ activity. Spriggs (39) reviewed the literature on soluble cytokine receptors produced by viruses, including pox viruses (e.g., smallpox), herpes viruses (including herpes simplex and potentially carcinogenic herpes viruses), and flu viruses (e.g., influenza A). Basler *et al.* (40) reported that Ebola virus induced the production of a protein that acts as an IFN inhibitor. It thus appears that a "virus-versus-host" defense system is based on inducing the production of free-circulating IFN receptors as well as other cytokines and IFN antagonists. In a series of 91 patients with cancer, free-circulating IFN-α/β type 1 receptors were found. The highest levels were observed in patients with adenocarcinomas, in the order: uterus > prostate > colon > ovary > breast cancer. They were all significantly higher than levels in 25 normal volunteers (Fig. 1; Ref. 41). A number of other types of cancer were also seen to have increased circulating IFN-α/β type 1 receptors, but, in each case, the number of patients studied was too small to draw definite conclusions. As individual categories, they were not included in Figure 1. Nevertheless, they were included in the "all neoplastic disorders" column. In these studies, IFN receptors were determined by the method of Novick *et al.* (25, 26, 42, 43).

As was indicated above, neoplastic tissue, the circulation of patients with neoplastic, autoimmune, or severe viral disorders contain high levels of PgE_2 which inhibits IFN synthesis and activity (1, 4–6).

Neoplastic tissue also contained high levels of cAMP-phosphodiesterases, which decrease IFN synthesis (5–7). Even though PgE_2 increases cAMP production, this effect is apparently offset by the high levels of cAMP phosphodiesterases.

In addition to these factors, a protein was isolated by 50% ammonium sulfate precipitation, which was a strong inhibitor of IFN activity, in the order: IFN-α > IFN-β > IFN-γ. However, no IFN inhibitory activity could be demonstrated against bovine and feline IFNs. This inhibitory activity was not caused by any nonspecific cytotoxicity to the test cells.

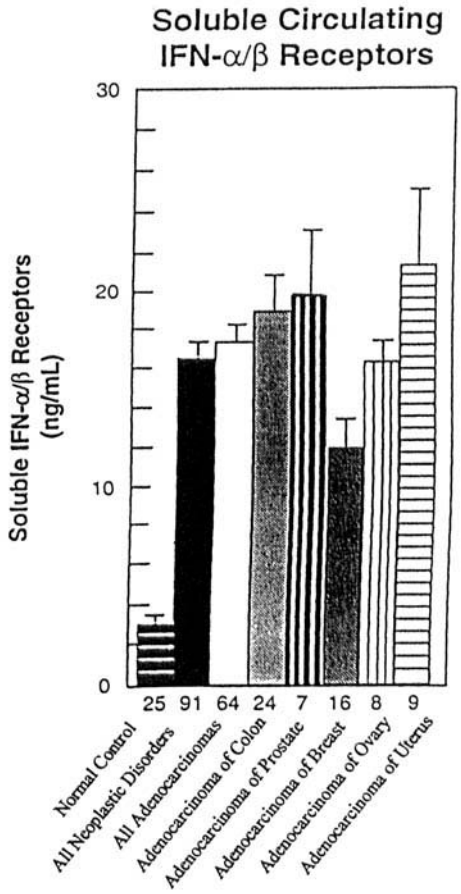

Figure 1. Free circulating IFN-α/β type 1 receptors in patients with cancer.

In an inhibitor assay, the loss of IFN antiviral activity could be caused by either direct complexing of the IFN inhibitor moiety with IFN or the inhibitor modifying the response of treated cells by either blocking the IFN receptors or in some way modifying the cell surface properties that subsequently blocks the expression of the IFN antiviral state. A series of experiments was done to distinguish between these possibilities. Test cells were pretreated with an IFN inhibitory preparation for 24 hrs. IFN antiviral activity was evaluated on these treated cells, in the absence or continued presence of IFN inhibitor. In all cases, a significant loss of IFN antiviral activity was seen, which suggests that IFN inhibitors can block IFN antiviral activity by complexing with IFN or by modifying the response of treated cells (3, 28, 30). The fact that the loss of IFN antiviral activity is caused by an inhibitor and not by some nonspecific factor(s) was shown when test cells were treated with a serial dilution of inhibitor preparation before antiviral assays. The loss of IFN antiviral activity was always proportional to the concentration of inhibitor used to pretreat the cells (3, 30).

It is well recognized that IFN enhances expression of NKC activity. IFN inhibitor has been shown to eliminate this IFN-mediated enhancement of NKC (31). nHuIFN-α enhances NKC activity in a standard ^{51}Cr-release assay. When lymphocytes were treated with inhibitory preparation prior to their use in NKC assay, no significant loss in NKC activity was seen. However, when effector cells were exposed simultaneously to IFN and IFN inhibitor, no significant increase in NKC activity was seen. This strongly suggests that the inhibitor is effective in blocking IFN-mediated enhancement of NKC (28, 30). The possible mechanism of how the inhibitor blocks IFN-mediated enhancement of NKC is not yet known.

Because IFNs are proteins, it is conceivable that inhibitory activity is associated with a high level of protease activity. Alternatively, the inhibitory activity could be due to antibody to IFN. Early evidence has shown that neither of these two possibilities is correct. In several independent experiments, no loss of inhibitory activity was seen when it was measured in the presence of different protease inhibitors, including phenylmethylsulfonyl fluoride, EDTA, and iodoacetamide. IFN inhibitor preparation was mixed with each of the protease inhibitors, alone or in combination, incubating the reaction mixture for 1 hr at 37°C, followed by dialysis against phosphate-buffered saline. After dialysis, the inhibitory activity was tested in a standard inhibitor assay. No loss of IFN inhibitory activity was seen (3, 30). The possibility that an inhibitor is an antibody to IFN was evaluated in two separate tests. If the inhibitor is an antibody to IFN, it should not lose its inhibitory activity at a low pH. The pH-treated (pH 2.2), dialyzed-inhibitor preparation lost >60% of its inhibitory activity, which suggests that it may not be an antibody to IFN. This possibility was further examined when a preparation containing IFN inhibitory activity was chromatographed on a phenyl-sepharose CL-4B column fully saturated with nHuIFN-α. If the inhibitor is an antibody to IFN, then the majority of inhibitor should be retained on phenyl sepharose CL-4B column fully saturated with nHuIFN-α. The chromatographic behavior of nHuIFN-α on phenyl-sepharose has been well documented (30). This ligand has strong affinity for nHuIFN-α and has been used for its purification. No IFN inhibitor activity was retained on a phenyl-sepharose CL-4B column fully saturated with nHuIFN-α, which further suggests that the IFN inhibitor is not an antibody to IFN (3, 30).

To overcome IFN resistance in many disease states, several attempts are being made to inhibit the inhibitors or to increase endogenous IFN production, also overcoming the effect of circulating inhibitors. In a preliminary series of studies (44), double-stranded RNA preparations were used to increase production of IFN. However, these preparations proved to be highly toxic *in vivo*. A series of thiolated polyI:polyC preparations was also explored. At 7.4% thiolation, a preparation termed polyI:polyMPC induced IFN-α, -β, and -γ in tissue culture and *in vivo* animal experiments; this was of minimal toxicity in animal studies. Further exploration of methods to overcome IFN inhibitory activity in certain disease states appears to be well justified.

There are at least 12 species of IFN-α, and a large number of additional IFNs were recently identified; 50 different IFNs are called "ultra-IFNs" (α, β, γ, and δ). Many artificial derivatives are also under study. Many of these have unique characteristics, greater potency, lesser potential for adverse effects, lesser immunogenicity, and cross-species activity. Sustained-release protein delivery methods and preparations for local intratumor injections are also under study. The effect of IFN inhibitory factors on these newly discovered agents are not yet known. Most of these studies are being carried out by the PBL Therapeutics Co. (Piscataway, NJ) and are unpublished. New IFNs are of special interest, because it is hoped that some will overcome recently discovered IFN resistance mechanisms. In Kaposi sarcoma, the K9 gene of KSH virus appears to encode an IFN regulatory factor that also binds P53, preventing its being phosphorylated and activated. Another related gene, ORF45, inhibits IFN production. The production of IFN regulatory factors may also down-regulate Fas-ligand expression, resulting in the inhibition of activation-induced cell death (45–48).

There are some pharmacologic incompatibilites of IFN therapy that should be briefly mentioned. Daubresser *et al.* (49, 50) reported that heparin inhibits some of the IFN-α activities, whereas the heparin-binding protamin molecule has stimulatory effect. Cortisol appears to decrease IFN-α activity (51). The further study of natural, disease-induced, and pharmacologic IFN antagonism and its relief appears to be well justified.

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1. Ikossi MG, Ambrus JL, Chadha KC. Regulation of in vitro human leukocyte interferon production: effect of prostaglandin synthetase and phosphodiesterase inhibition. Res Commun Chem Pathol Pharmacol 54:379–384, 1986.

2. Chadha KC, Ambrus JL, Halpern J, Khalil M, Sayyid S, Piver MS, Hreshchyshyn MM. The interferon system in carcinoma of the cervix: effect of radiation and chemotherapy. Cancer 67:87–90, 1991.

3. Chadha KC, Ambrus JL, Ikossi MG. Interferons and interferon inactivators in AIDS, ARC and in cancer patients. In: Cantell K, Schellekens H, Eds. The Biology of the Interferon System, 1986. Proceedings of the 1986 ISIR-TNO Meeting on the Interferon System. September 7–12, 1986, Helsinki, Finland: Martinus Nijoff, pp423–427, 1987.

4. Ambrus JL, Chadha KC, Anthone S, Anthone R, Sayyid S. The alpha-interferon system in patients undergoing renal transplantation and treated with cyclosporine and prednisone. Surg Gynecol Obstet 176: 588–590, 1993.

5. Stefanovich V, Ambrus JL, Ambrus CM, Karakousis C, Takita H. Cyclic nucleotide phosphodiesterases in normal and malignant human tissue. J Med 12:243–250, 1981.
6. Ambrus JL, Stoll JL, Klein E, Karakousis CP, Stadler S. Increased prostaglandin E_2 and cAMP phosphodiesterase levels in Kaposi's sarcoma: a virus-against-host defense mechanism. Res Commun Chem Pathol Pharmacol 78:249–252, 1992.
7. Attallah A, Lee JB, Ambrus JL, Ambrus CM, Karakousis CP, Takita H. Prostaglandin E_2 production by human tumors: defense mechanism against the host. Res Commun Chem Pathol Pharmacol 43:195–201, 1984.
8. Stadler I, Kapui Z, Ambrus JL. Study on the mechanisms of action of sodium meclofenamic acid (Meclomen), a "double inhibitor" of the arachidonic acid cascade. J Med 25:371–382, 1994.
9. Mahafzah M, Stadler S, Ambrus JL, Chadha KC. Effect of meclomen on production and action of human interferon alpha. Acta Virol 34: 27–36, 1990.
10. Stadler S, Chadha, KC, Nakeeb S, Toumbis C, Butsch J, Mathur N, Munschauer F, Vladutiu A, Ambrus JL. Pentoxifylline and meclofenamic acid treatment reduces clinical manifestations in a murine model of AIDS. J Pharmacol Exp Ther 268:10–13, 1994.
11. Aszalos A, Grimley PM, Balint E, Chadha KC, Ambrus JL. On the mechanisms of action of interferons: interaction with nonsteroidal, anti-inflammatory agents pentoxifylline (Trental) and cGMP and inducers. J Med 22:255–271, 1991.
12. Biddle W, Leong SS, Horoszewicz J, Ambrus JL. Potentiation of the cell growth inhibitory effect of β-interferon by mopidamole. Proc Soc Exp Biol Med 177:487–490, 1984.
13. Sikora K, Ed. Interferon and Cancer. New York: Plenum Press, 1983.
14. Gutterman J. Cytokine therapeutics: lessons from interferon-α. Proc Natl Acad Sci U S A 91:1198–1205, 1996.
15. Pitha PM. Introduction of interferon's connection to cancer. Semin Cancer Biol 10:69–72, 2000.
16. Einhorn S, Grander D. Why do so many cancer patients fail to respond to interferon therapy? J Interferon Cytokine Res 16:275–281, 1996.
17. Einhorn S, Strander H. Interferon treatment of human malignancies: a short review. Med Oncol Tumor Pharmacother 10:25–29, 1993.
18. Ikossi-O'Connor MG, Ambrus JL, West T, Lillie MA, Milgrom H, Chadha KC. Interferon and interferon inactivators in patients with acquired immune deficiency syndrome and Kaposi's sarcoma. Res Commun Chem Pathol Pharmacol 45:271–277, 1984.
19. Tsantakis G, Schizas N, Harvedaki M. Interferon inhibitors and/or inactivators in sera from patients with neoplasias. Arch Immunol Ther Exp 41:335–337, 1993.
20. Cesario T, Vaziri N, Tilles J. Inactivators of fibroblast interferon found in human serum. Infect Immun 24:851–855, 1979.
21. Karmaniolas KD, Papalampros TS, Papavassiliou ED, Liatis STh, Kalantzis N. Detection of interferon inhibitors or antagonists in gastrointestinal malignancies. Hepatogastroenterology 45:2244–2247, 1998.
22. Ambrus JL, Poiesz BJ, Lillie MA, Stadler S, DiBernardino L, Chadha KC. Interferon and interferon inhibitor levels in patients infected with varicella-zoster virus, AIDS, ARC, Kaposi's sarcoma and normal individuals. Am J Med 87:405–407, 1998.
23. O'Connor MGI, Chadha KC, Lillie MA, Stadler S, Bernstein Z, Zucker-Franklin D, Ambrus JL. Interferon inactivator(s) in patients with AIDS and AIDS-related Kaposi's sarcoma. Am J Med 81:783–786, 1986.
24. Harmon MW, Greenberg SB, Couch RB. Effect of human nasal secretions on the antiviral activity of human fibroblast and leukocyte interferon. Proc Soc Exp Biol Med 152:598–602, 1976.
25. Novick D, Engelmann H, Wallach D, Rubinstein M. Soluble cytokine receptors are present in normal human urine. J Exp Med 170:1409–1414, 1989.
26. Novick D, Cohen B, Rubinstein M. Soluble interferon-alpha receptor molecules are present in body fluids. Fed Eur Biochem Soc 314:445–448, 1992.
27. Havredaki M, Barona F. Variations of interferon inactivators and/or inhibitors in human serum and their relationship to interferon therapy. Jpn J Med Sci Biol 38:107–111, 1985.
28. Ambrus JL, Ambrus JL Jr, Chadha S, Novick D, Rubinstein M, Gopalakrishnan B, Bernstein Z, Priore R, Chadha KC. Mechanism(s) of interferon inhibitory activity in blood from patients with AIDS and patients with lupus erythematosus with vasculitis. Res Commun Mol Pathol Pharmacol 96:255–265, 1997.
29. Ambrus JL, Ambrus JL Jr, Chadha KC. Interferon inhibitors in lupus erythematosus. N Engl J Med 319:582–583, 1986.
30. Chadha KC, Ambrus JL, Stadler S, Ambrus JL Jr. Interferon inhibitor in the blood of patients with systemic lupus erythematosus. J Biol Regul Homeost Agents 5:1–9, 1991.
31. Ambrus JL, Chadha KC, Ambrus JL Jr. Interferon inhibitors in Wegner's granulomatosis. Ann Intern Med 114:606–607, 1991.
32. Chadha KC, Ambrus JL, Halpern J, Khalil M, Piver MS, Hreshchyshyn MM. The interferon system in gynecologic cancer: effect of radiation and chemotherapy. Cancer 67:87–91, 1991.
33. Finter NB. Dye uptake method of assessing viral cytopathogenicity and their application to interferon assays. J Gen Virol 5:419–427, 1969.
34. Payne J, Nair MPN, Ambrus JL, Chadha KC. Mild hyperthermia modulates biological activities of interferons. Int J Hyperthermia 16(6): 492–507, 2000.
35. Ambrus JL, Chadha KC, Ambrus JL Jr. Interferon inhibitors. Ann Intern Med 114:606–607, 1991.
36. Hardy MP, Owczarek CM, Trajanovska S, Liu X, Kola I, Hertzog PJ. The soluble murine type 1 interferon receptor Ifnar-2 is present in serum, is independently regulated, and has both agonistic and antagonistic properties. Blood 97:473–482, 2001.
37. Moosmayer D, Gerlach E, Hauff R, Becker P, Brocks B, Pfizenmaier K. A bivalent immunoadhesion of the human interferon-gamma receptor is an effective inhibitor of IFN-gamma activity. J Interferon Cytokine Res 15:1111–1115, 1995.
38. Ozmen L, Gribaudo G, Fountoulakis M, Gentz R, Landolfo S, Garotta G. Mouse soluble IFN-gamma receptor as IFN-gamma inhibitor. J Immunol 150:2698–2705, 1993.
39. Spriggs MK. Poxvirus-encoded soluble cytokine receptors. Virus Res 33:1–10, 1994.
40. Basler CF, Wang X, Muhlberger E, Volchkov V, Paragas J, Klenk H-D. The Ebola virus VP35 protein functions as a type 1 IFN antagonist. Proc Natl Acad Sci U S A 97:12289–12294, 2000.
41. Ambrus JL, Dembinski W, Ambrus JL Jr, Sykes DE, Akhter S, Kulaylat, MN, Islam A, Chadha KC. Free interferon-α/β receptors in the circulation of patients with adenocarcinomas. Cancer 98:2730–2733, 2004.
42. Novick D, Engelmann H, Leitner O, Rubinstein M. Monoclonal antibodies to the soluble human IL-6 receptor: affinity purification, ELISA, and inhibition of ligand binding. Hybridoma 10:137–146, 1991.
43. Novick D, Engelmann H, Wallach D, Leitner O, Revel M, Rubinstein M. Purification of soluble cytokine receptors from normal human urine by ligand-affinity and immunoaffinity chromatography. J Chromatogr 510:331–337, 1990.
44. Ambrus JL, Islam A, Akhter S, Kulaylat M, Sykes D, Dembinski W, Bardos TJ, Aradi J, Chadha P, Chadha KC. New potent interferon inducing agents: Thiolated poly (I). Poly (MPC). Proceedings of Spring Clinical Day, State University of New York at Buffalo, pp1–10, 1999.

45. Nakamura H, Li M, Zarycki J, Jung JU. Inhibition of P53 tumor suppressor by viral interferon regulatory factor. Virology 75:7572–7582, 2001.
46. Zhu FX, King SM, Smith EJ, Levy DE, Yuan Y. A Kaposi's sarcoma–associated herpesviral protein inhibits virus-mediated induction of type 1 interferon by blocking IRF-7 phosphorylation and nuclear accumulation. Proc Natl Acad Sci U S A 99:5573–5578, 2002.
47. Poole LJ, Yu Y, Kim PS, Zheng QZ, Pevsner J, Hayward GS. Altered patterns of cellular gene expression in dermal microvascular endothelial cells infected with Kaposi's sarcoma–associated herpesvirus. J Virol 76:3395–3420, 2002.
48. Kirchoff V, Wong S, St JS, Pari GS. Generation of a life-expanded rhesus monkey fibroblast cell line for the growth of rhesus rhadinovirus (RRV). Arch Virol 147:321–333, 2002.
49. Daubener W, Nockemann S, Gubsche M, Hadding U. Heparin inhibits the antiparasitic and immune modulatory effects of human recombinant interferon-gamma. Eur J Immunol 25:688–692, 1995.
50. Daubener W, Gutsche M, Nockemann S. Protamine enhances the activity of human recombinant interferon-γ. J Interferon Cytokine Res 16:531–536, 1996.
51. Spath-Schwalbe E, Porzsolt F, Digel W. Elevated plasma cortisol levels during interferon-γ treatment. Immunopharmacology 17:141–145, 1989.