

Alpha Interferon in the Treatment of Hematologic Malignancies

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The interferons are an important first member of a family of biologic response-modifiers used in treating human malignancies. Activities associated with the interferons include inhibition of viral replication, influence on cellular protein production, direct antiproliferative effects, and a variety of modulatory effects on the immune response. These regulatory functions of interferon underlie the interest in its use as an anticancer agent. Alpha interferon is the most extensively studied interferon species. Although antitumor activity has been seen both *in vitro* and *in vivo* in some solid malignancies, the most impressive responses have occurred in the hematologic malignancies. More than 90 percent of patients with hairy cell leukemia have a sustained recovery of their peripheral blood cell counts with alpha interferon therapy. Approximately 50 percent of patients with low-grade non-Hodgkin's lymphoma and cutaneous T cell lymphoma demonstrate a response to alpha interferon. More than 80 percent of patients with chronic myelogenous leukemia have a response to alpha interferon, and in one study, nearly half of the patients with response had complete suppression of the Philadelphia chromosome clone on at least one examination. Ongoing clinical trials are addressing such issues as optimal dosage, duration of alpha interferon therapy, and combinations of alpha interferon with other biologic agents, chemotherapy drugs, and radiation.

Interferon was the term originally applied to a soluble factor that was recognized by its ability to induce interference against viral infection of chick chorioallantoic membrane by influenza A virus [1]. It has subsequently been shown to be a family of closely related proteins and glycoproteins that, in addition to antiviral activity, are potent regulators of cellular function and structure and possess direct antiproliferative activities. These latter properties underlie the current interest in interferon as an anticancer agent.

Three major species of human interferon are recognized and designated alpha interferon, beta interferon, and gamma interferon [2] (Table I). Alpha interferon is produced by leukocytes (B cells, T cells, null cells, and macrophages) upon exposure to B cell mitogens, viruses, foreign cells, or tumor cells. Beta interferon is produced by fibroblasts upon exposure to viruses or foreign nucleic acids. Gamma interferon is produced by T lymphocytes upon stimulation with T cell mitogens, specific antigens, or interleukin 2 [3]. By use of recombinant DNA techniques, complete nucleotide sequences for alpha, beta, and gamma interferons have been defined, and amino acid sequences have been derived.

The genes recognized to code for alpha interferon have been assigned to chromosome 9 [4]. Sixteen distinct sequences for alpha interferon have been described [4]. Each is approximately 166 amino acids

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TABLE I Interferons in Clinical Use

Type	Subtype* (new nomenclature)	Source	Purity (percent)	Amino Acid Differences	
Alpha	Leukocyte (IFN-alpha [LE])	Leukocytes from normal blood	<1†		
	Lymphoblastoid (IFN-alpha-N1) Wellferon (Burroughs Wellcome)	Lymphoblastoid (Namalva) cells in culture	<1†		
	Recombinant alpha-2 (IFN-alpha-2b) Intron-A (Schering)	Transformed E. coli	>95	Arginine at position 23, deletion at position 44 when compared with other alpha subtypes	
	Recombinant alpha-A (IFN-alpha-2a) Roferon-A (Hoffman-La Roche)	Transformed E. coli	>95	Lysine at position 23, deletion at position 44	
	Recombinant alpha-D (IFN-alpha-D)	Transformed E. coli	>95	29 variations from alpha-A	
	Recombinant alpha-2 arg (IFN-alpha-2c)	Transformed E. coli	>95	Arginine at position 23, arginine at position 34	
	Beta	Fibroblast (IFN-beta)	Fetal foreskin fibroblast in culture	<1†	
		Recombinant beta cys (rIFN-beta cys)	Transformed E. coli	>95	Cysteine at position 17
Recombinant beta ser (rIFN-beta ser)		Transformed E. coli	>95	Serine at position 17	
Gamma	Immune (IFN-gamma)	T lymphocytes from normal blood	<1†		
	Recombinant gamma (rIFN-gamma)	Transformed E. coli	>95		

* New nomenclature was proposed at a joint meeting of the World Health Organization and USAN council in May 1985.

† These crude preparations can be purified to near homogeneity (see text).

in length with an additional 20 amino acid secretory peptide present on the amino-terminal end. The human genes differ by approximately 10 percent in nucleotide sequence and 15 to 20 percent in amino acid sequence [5]. Two recombinant human interferons, alpha A and alpha D, make up more than 60 percent of interferons present after buffy coat stimulation and have been extensively studied [6]. Although they possess different antiviral and antiproliferative activity in vitro, similar in vivo effects on immune effector cells have been observed [6]. The alpha interferon used in the first human clinical trials was obtained from Sendai virus-stimulated buffy coat leukocytes and represented 1 percent purity (10^6 units/mg protein)* [7]. Refinement in purification methods by use of high-performance liquid chromatography, two-dimensional polyacrylamide gel electrophoresis, and immunoaffinity chromatography has allowed purification to homogeneity (10^8 units/mg protein) [8–10]. The use of recombinant DNA techniques with splicing of the alpha interferon gene into *Escherichia coli* has further allowed for pure single-species alpha interferon in larger quantities.

Unlike alpha interferon, only a single protein species has been identified for both beta and gamma interferon

* One unit of interferon is roughly the amount that reduces viral replication in tissue culture by half.

[5]. Beta interferon consists of 166 amino acids with 45 percent homology of nucleotides and 29 percent amino acids compared with alpha interferon [5]. Gamma interferon consists of 146 amino acids and has approximately 12 percent amino acid sequence homology compared with alpha interferon [11]. Gamma interferon may exist in biologic fluids in a dimeric form [12].

Industrial-scale production of beta and gamma interferon has only recently been accomplished, and clinical trials are limited in number. Alpha interferon, however, has been extensively studied for the past decade in both basic science and clinical research, and it is among the most potent biologic agents ever administered to man. Although antitumor activity has been seen both in vitro and in vivo in some solid malignancies (breast cancer, renal cell cancer, Kaposi's sarcoma, bladder cancer, ovarian cancer, and melanoma) [13,14], the most impressive responses have occurred in the hematologic malignancies. A review of these results and proposed mechanisms of action are presented.

CLINICAL EXPERIENCE

A summary of clinical trials using alpha interferon for the hematologic malignancies is presented in Table II. Some reported trials have used highly purified preparations (10^8 units/mg protein), whereas others have used crude preparations of alpha interferon (10^6 units/mg protein). Impuri-

TABLE II Clinical Trials with Alpha Interferon in Hematologic Malignancies

Reference	Tumor	Number of Evaluable Patients	Response Rates			Percent Total Response*
			Complete	Partial	Minor	
[18-27]	Hairy cell leukemia [†]	158	22	86	44	96
	Non-Hodgkin's lymphoma					
[29-35]	Low-grade	92	9	30	6	42
[29,31,33,35,36]	Intermediate- and high-grade	36	1	4	2	14
[33]	Hodgkin's disease	8	0	0	2	0
[37,38]	Cutaneous T cell lymphoma	20	2	7	2	45
[35,40-44]	Chronic lymphocytic leukemia	67	0	12		18
[35,46-53]	Multiple myeloma	224	3	41 [‡]		20
[56-58]	Chronic myelogenous leukemia	68	8	40	7	81
[59]	Essential thrombocythemia	4	3	0		75
[60-64]	Acute leukemia	62	0	19 [§]		31

* Percent total response = complete responses + partial responses ÷ number of evaluable patients.

[†] Complete response means absence of hairy cells in the bone marrow and normalization of peripheral blood white cells, platelets, and erythrocytes. Partial response means a normalization of peripheral blood white cells, platelets, and erythrocyte counts and greater than 50 percent reduction in hairy cells in the bone marrow. Minor response generally means improvement in hemoglobin to more than 10 g/dl or improvement in platelets to more than 100,000 cells/ μ l or improvement in neutrophils to more than 1,000 cells/ μ l. Percent total response for hairy cell leukemia includes minor responses.

[‡] Complete response and partial response not available from all trials, percent total response includes all responses.

[§] Most responses were of short duration.

ties in the latter include albumin, transferrin, and additional lymphokines. Despite these contaminants, the toxicities and antitumor responses seen with both preparations have been similar. The major side effects have involved a flu-like illness (fever, chills, muscle aches, headache, gastrointestinal upset, and fatigue). The onset of fever is generally four to eight hours after administration with a duration of approximately 12 hours. With repeated administration, tachyphylaxis to fever usually occurs, but fatigue and anorexia increase with dosage and duration of treatment and remain the usual dose-limiting toxicities. Other reported side effects include dose-related myelosuppression, elevated transaminase concentrations, paresthesias, anosmia, somnolence, confusion, and impotence in men. One occurrence of interstitial nephritis has been reported [15], and elevation of hepatic transaminase levels was the dose-limiting toxicity in another study [16]. All of these toxicities are reversible with cessation of drug.

HAIRY CELL LEUKEMIA

Hairy cell leukemia is a well-characterized lymphoproliferative disorder in which cells with lymphoid morphology and villous cytoplasmic projections infiltrate the bone marrow, blood, and reticuloendothelial system. It is of B cell origin and usually presents with cytopenias [17]. The disease is often indolent, with a median age of onset 50 years and a 5:1 male-to-female ratio. Standard initial therapy is splenectomy, which often restores hematologic parameters to normal; however, most of these patients have a relapse weeks to years after splenectomy. Treatment of relapses has been generally poor with standard

cytotoxic agents. Excellent responses were reported [18] in seven patients with hairy cell leukemia (three complete and four partial responses) treated with crude alpha interferon. Similar data have been reported by a number of investigators using recombinant alpha interferon. Response rates have been comparable with recombinant preparations after therapy three times a week or daily with dosages ranging from 3 to 6 $\times 10^6$ units intramuscularly or subcutaneously [18-27]. Although the initial report suggested that complete responses were frequent, this has not been confirmed (of 158 responses reported, only 22 were complete) [18-27]. More important, however, is that virtually all of the patients with response demonstrated normalization of peripheral blood cell counts, which was maintained while they were receiving interferon therapy. Many of these patients had no prior therapy, including splenectomy. In patients with response, disease has not been reported to become refractory to alpha interferon; many patients have been followed for more than three years. In addition, improvement in natural killer activity and immunologic surface markers parallels the hematologic recovery [24]. In a recent study [20], interferon treatment was discontinued in 25 patients after 12 months of treatment. In eight of the 25 patients, a relapse occurred at a median of six months after cessation of treatment and resulted in reinduction of remission in five of the eight patients who have completed three months of therapy. Studies to assess low-dose (3 to 4 $\times 10^6$ units) compared with ultra-low-dose (0.3 to 0.4 $\times 10^6$ units) alpha interferon are currently underway. Phase III trials in which patients with newly diagnosed hairy cell leukemia are randomly assigned to undergo splenectomy or receive

alpha interferon treatment are also underway. Although hairy cell leukemia accounts for fewer than 2 percent of all cases of leukemia, its response to alpha interferon makes it an ideal disease to study the putative mechanisms of activity that are addressed next.

NON-HODGKIN'S LYMPHOMA AND HODGKIN'S DISEASE

The histologic classification of non-Hodgkin's lymphoma was recently reformulated from the commonly used Rapaport system. On the basis of prognosis and morphology, the histologic types of malignant lymphoma have been grouped into low-, intermediate-, and high-grade malignancy under the Working Formulation [28]. Although many chemotherapy agents produce responses, low-grade non-Hodgkin's lymphoma is not curable with currently available treatment. This, in combination with the indolent nature of the disease, leads to multiple episodes of treatment and relapse; eventually the patient dies from unrelated causes, toxicity of therapy, progressive disease, or emergence of a more aggressive histologic subtype. The low-grade non-Hodgkin's lymphoma have shown responses to alpha interferon [29-35]. Early results with crude alpha interferon preparations reported responses to alpha interferon in four of seven patients [29,30]. In the largest series reported to date [31], previously treated patients received recombinant leukocyte alpha interferon at a dose of 50×10^6 units/m² of body surface area intramuscularly three times a week. Thirteen responses were obtained (four complete and nine partial responses) among 24 evaluable patients, with a median duration of response of eight months. Alpha interferon in combination with standard cytotoxic agents is currently under investigation as first-line therapy.

Alpha interferon has shown less effectiveness in the intermediate- and high-grade lymphomas. Thirty-six patients have been treated with both crude and recombinant alpha interferon, and five responses were reported [29,31,33,35,36]. Further study of alpha interferon in unfavorable non-Hodgkin's lymphoma may be warranted to establish which patient subgroups might benefit from treatment.

Eight patients with advanced refractory Hodgkin's disease have been treated with crude alpha interferon [33]. Only two brief minor responses were reported. In a recent study, however, with recombinant alpha interferon, approximately 30 percent of patients with advanced refractory Hodgkin's disease have shown response (E. Bonnem, personal communication).

CUTANEOUS T CELL LYMPHOMA

Cutaneous T cell lymphoma (mycosis fungoides and the Sézary syndrome) is a non-Hodgkin's lymphoma characterized by a malignant proliferation of mature helper T lymphocytes that presents with skin infiltration and an

indolent clinical course. Effective therapies include topical mechlorethamine, psoralen plus ultraviolet light, total skin electron-beam irradiation, and systemic chemotherapy. Unfortunately, prolonged disease-free survival has been reported only rarely with these therapies, and the best response rates for advanced disease are reported to be about 25 percent with short duration of response [37]. Responses in nine of 20 patients (two complete and seven partial) with advanced stages of disease refractory to prior therapy were observed [38] using recombinant alpha interferon at an intramuscular dosage of 50×10^6 units/m² body surface area three times a week. Responses, defined as at least a 50 percent decrease in the sum of perpendicular measurements of malignant lesions lasting at least one month, occurred within four weeks of therapy and lasted three months to more than 25 months. Extracutaneous responses also occurred. A decrease in the size of large lesions by more than 90 percent occurred in a number of patients, suggesting that alpha interferon is the best single agent for cutaneous T cell lymphoma.

CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia is a hematologic malignancy characterized by proliferation and accumulation of relatively mature-appearing lymphocytes. Most patients have a clonal proliferation of B lymphocytes [39]. Chronic lymphocytic leukemia typically occurs in persons over 50 years (median age 60 years) and affects men more than women at a ratio of 2:1 [39]. The disease is usually stable over months to years, but transformation to a more aggressive disease state does occur. Alkylating agents, radiation therapy, and corticosteroids are commonly used to treat patients, although few data show that survival is substantially improved. In a number of early studies crude alpha interferon preparations were reported to be effective in patients with advanced chronic lymphocytic leukemia [35,40,41]. In a phase II trial of recombinant alpha interferon, 18 patients were treated with both high-dose (50×10^6 units/m² intramuscularly) and low-dose (5×10^6 units/m² intramuscularly) recombinant alpha interferon three times a week [42]; only two brief responses were reported. Five patients appeared to have an acceleration of disease while receiving recombinant alpha interferon. This low response rate was confirmed by a number of investigators [32,33,41,43,44]. It is in marked contrast to responses in patients with chemotherapy-refractory low-grade non-Hodgkin's lymphoma and hairy cell leukemia as previously described, and the possible mechanism for this will be addressed.

MULTIPLE MYELOMA

Multiple myeloma is a disease of uncontrolled proliferation of malignant plasma cells in the marrow; it manifests clinically by tumor formation, osteolysis, hemopoiesis, hypogammaglobulinemia with a paraprotein monoclonal

spike, and renal disease. The mean age at the time of diagnosis is 62 years. Multiple myeloma responds initially to a variety of chemotherapeutic agents; however, once it becomes refractory to first-line therapy, further responses are difficult [45]. A number of trials with crude and recombinant alpha interferon in patients with multiple myeloma have been reported [46–53]. In a pilot study, four previously untreated patients were treated daily with crude alpha interferon (3×10^6 units intramuscularly). All patients demonstrated durable responses (two complete and two partial) lasting three to 19 months [46]. This study was extended into a prospective randomized trial comparing crude alpha interferon (3×10^6 units intramuscularly daily) with melphalan/prednisone on a six-week schedule. Fifty-three patients were allotted to melphalan/prednisone and 62 patients to alpha interferon treatment. Total response rate was higher in the melphalan/prednisone group (41 percent) than in the interferon group (14 percent) ($p < 0.05$) (response defined as a greater than 50 percent decrease in paraprotein) [47].

Recombinant alpha interferon has been administered in a number of trials [49–53]. Dosages ranged from 2×10^6 units/m² to 100×10^6 units/m² daily. Only 22 of 122 previously treated patients had responses, compared with seven of 19 untreated patients. Of note is a recent observation of synergy between alpha interferon and high-dose chlorambucil in patients with refractory myeloma [54]. Further trials of combination alkylating agent and interferon are ongoing.

CHRONIC MYELOGENOUS LEUKEMIA

Chronic myelogenous leukemia is a neoplastic disease characterized by clonal proliferation of a myeloid stem cell. A unique chromosomal translocation, the Philadelphia chromosome, is present in about 90 percent of patients. The peak age of onset is 40 years. The clinical manifestation of the disease relates to accumulation of large numbers of immature and mature granulocytic cells in the blood and abdominal viscera. In most patients, the proliferation of the hematopoietic cells can be suppressed for one to four years with cytotoxic agents, but acute leukemia or a blast crisis develops in more than 80 percent of patients [55]. In the acute phase, therapeutic agents including those useful in the treatment of acute leukemia are ineffective. Fifty-one patients with chronic myelogenous leukemia were treated with 3 to 9×10^6 units daily of crude (10^6 units/mg protein) alpha interferon intramuscularly [56]. Forty-one of the patients demonstrated response to therapy, with complete (36 patients) or partial response (five patients) in the peripheral blood. Patients with response showed a gradual decrease of spleen size to normal and decrease in bone marrow cellularity. Suppression of the Philadelphia chromosome occurred in varying degrees in 20 of 51 patients and was complete in two patients. Successful lowering of platelet counts in nine patients (all previously treated) with severe

symptomatic thrombocytosis has also been demonstrated [57] with crude alpha interferon. A recent study using 5×10^6 units/m² of recombinant alpha interferon daily demonstrated 13 hematologic remissions and one partial hematologic remission among 17 patients [58]. In six of the patients with hematologic remission, there was complete suppression of the Philadelphia clone on at least one examination. Although these are very exciting data, they are preliminary and require confirmation.

ESSENTIAL THROMBOCYTHEMIA

Essential thrombocythemia is a myeloproliferative disease defined by a platelet count generally in excess of $10^6/\mu\text{l}$, megakaryocyte hyperplasia in bone marrow, and absence of a predisposing cause (i.e., Philadelphia chromosome, increased red cell mass, infection, or iron deficiency). Essential thrombocythemia usually appears between the ages of 50 and 70 years. The major morbidity of the disease is bleeding and thrombosis with a 50 percent 5-year survival rate. Several agents (³²P, L-phenylalanine mustard, busulfan, uracil mustard, and hydroxyurea) have been effective in lowering platelet counts. Recombinant alpha interferon has been administered to four previously untreated patients with essential thrombocythemia at a daily dosage of 5 to 10×10^6 units intramuscularly for 30 days [59]. Platelet counts returned to normal in three of the four patients. Maintenance alpha interferon therapy twice a week was given after 30 days, and patients were followed for up to 80 days without relapse. Because no known leukemogenic potential exists for alpha interferon, it may become a useful initial treatment of essential thrombocythemia.

ACUTE LEUKEMIA

Acute leukemia is a malignant stem cell disorder characterized by uncontrolled growth of poorly differentiated lymphoblasts. Early studies with crude alpha interferon were reported to produce responses in six of seven patients with acute lymphoblastic leukemia and two of three with acute nonlymphoblastic leukemia at daily dosages of 0.5 to 5×10^6 units/kg intravenously for two weeks to two months. In phase I and II trials [62,63], 53 patients were treated with partially pure lymphoblastoid alpha interferon (5 to 200×10^6 units/m² daily for 10 days). Five of 33 patients with leukemia experienced significant (80 to 99 percent) decreases in circulating blast counts, but bone marrow pathologic studies revealed only three patients with any degree of improvement in bone marrow infiltration (two transiently and one for three months). Recombinant alpha interferon (25 to 100×10^6 units daily for seven days) was administered to 13 heavily pretreated patients with only two minimal responses [64]. Alpha interferon in high doses has had limited effectiveness for management of patients with acute leukemia. The role of lower dose alpha interferon has yet to be determined.

TABLE III Cellular Events after Treatment with Alpha Interferon

Intracellular protein changes
Increased 2'-5'-oligoadenylate synthetase
Increased protein kinase activity
Direct antiproliferative effects
Antiproliferative effect on tumor cell lines
Antiproliferative effect on murine tumors in vivo
Antiproliferative effects on transplanted tumors in nude mice
Immunomodulatory activities
Enhancement (low dose) or suppression (high dose) of natural killer activity
Augmentation of antibody dependent cellular cytotoxicity
Enhancement of tumoricidal activity of macrophages
Regulation of antibody production in B cells
Enhancement of cytotoxic phase of mixed lymphocyte culture
Depression of lymphoproliferative phase of mixed lymphocyte culture
Increased expression of cell surface antigens, HLA-A, B, C, and B ₂ microglobulin
Decreased oncogene expression

MODE OF ACTION

The effect of interferon at the cellular level is initiated by binding of the interferon molecule to a cell surface membrane receptor [65]. Competitive binding studies indicate that alpha and beta interferon interact with one cell surface receptor, whereas gamma interferon may interact with another receptor [65]. After binding to the cell surface membrane, human interferon is rapidly internalized and degraded [66]. Whether this internalization is required for the biologic responses to interferon has not been resolved. Similar to several polypeptide hormones and their target cells, a down-regulation of interferon receptors after exposure of cells to interferon occurs [66].

Direct and indirect mechanisms of the anticancer activity of interferon probably result from a number of different mechanisms, including induction of several intracellular proteins, enhancement of immune effector cells, and changes in cellular surface structure (Table III). Two enzymes appear to play a major role in interferon activity. Treatment of cells in culture with interferon results in an increase in 2'-5'-oligoadenylate synthetase [67,68]; studies suggest that this response represents the induction of a gene that is subject to control by interferon [69]. 2'-5'-oligoadenylate synthetase is capable of synthesizing a novel series of oligonucleotides, 2'-5'-oligoadenylates, in the presence of double-stranded RNA and ATP. These oligonucleotides range from 2 to 15 in length and are collectively referred to as 2'-5'A. 2'-5'A in turn activates a latent endoribonuclease that is capable of cleaving both viral and host RNA (messenger RNA and ribosomal RNA) effectively inhibiting transcription and translation [65]. 2'-5'A introduced into normal and neoplastic cells appears to inhibit both protein and DNA synthesis [70]. The second enzyme activated by exposure of cells to interferon is a

protein kinase capable of phosphorylating peptide eukaryotic initiation factor (eIF-2 alpha) and ribosome-associated protein P₁ [65,71]. Recent observations suggest that the interferon-induced protein kinase is protein P₁ [71]. The net result of the kinase activation is the inhibition of peptide chain initiation. The exact role of these observations in relation to anticancer activity remains undetermined. Preliminary data exist correlating the levels of induced 2'-5'-oligoadenylate synthetase with alpha interferon administration [72]; however, correlation with anti-tumor activity has not been made [73].

ANTIPROLIFERATIVE EFFECT

Alpha interferon has antiproliferative effect on some malignant tumor cells. Dose-dependent in vitro inhibition of hematologic cell lines using alpha interferon has been shown in Burkitt's lymphoma, lymphocytic lymphoma, acute myelogenous leukemia, chronic myelogenous leukemia, chronic lymphocytic leukemia, and multiple myeloma [74-78]. Interestingly, in comparative antiproliferative studies, alpha interferon has shown a greater inhibitory effect in cells of hematopoietic origin than either beta or gamma interferon, using both crude and recombinant interferons [75,76,79]. Of note, noncycling tumor cells (G₀-G₁) appear to be a more sensitive target for the antiproliferative activity of human interferon [80,81].

Crude murine alpha interferon preparations have been shown to inhibit the growth of transplantable tumors of diverse origins (melanoma, friend leukemia, osteogenic sarcoma, Lewis lung, Ehrlich ascites) [82-85]. In support of a direct antiproliferative effect are studies of transplanted human tumors in immunodeficient nude mice in which immunomodulatory effects of administered human alpha interferon are minimal [86,87]. Dose-dependent growth inhibition is observed in these models and persists only for the duration of treatment [86,87]. Evidence for direct antiproliferative effect in human trials is suggested in cutaneous T cell lymphoma. Four of 10 patients who had had a relapse while receiving a 10 percent maintenance dosage demonstrated responses after reescalation to 100 percent dosage [37].

IMMUNOMODULATORY ACTIVITY

Immunomodulatory activities of interferon are also of considerable interest and may possibly play a role in the anticancer effect. The first evidence of this indirect effect of interferon was demonstrated when mice inoculated with L1210 cells derived from an interferon-resistant clone were still protected by daily interferon treatment [88]. Because the resistant cells did not revert to interferon-sensitive cells in vivo, these experiments were interpreted as suggesting that an antitumor effect was mediated by the host, rather than by a direct effect on cell multiplication. Subsequently it has been shown that alpha interferon can enhance as well as suppress cell-mediated

and humoral immune responses that are believed to play an active role in tumor surveillance.

Natural Killer Activity and Antibody-Dependent Cellular Cytotoxicity. Natural killer cells are a heterogeneous population of lymphocytes that are cytotoxic against several cell types and tumors in vitro [89]. Morphologically, human natural killer cells are identified as large granular lymphocytes. Direct evidence exists that natural killer cells inhibit in vivo tumor growth in animals; inhibition in humans has thus far been indirect and less consistent [90]. In in vitro systems, human natural killer cell cytolytic activation is consistently enhanced in the presence of both crude and pure alpha interferon [91–93]. There is conflicting evidence regarding the natural killer cell-activating effects of interferon in human therapy. Using both crude and pure alpha interferon, many clinical studies have documented interferon-induced increases in natural killer cell activity [94,95], whereas other investigators reported a lack of effect on natural killer activity or occasional depression of natural killer cytolytic activity by interferon [96,97]. To define the parameters governing this effect, purified lymphoblastoid interferon was given in one study in six dosages, ranging from 10^5 to 3×10^7 units intramuscularly per week to cancer patients [98]. A negative correlation between the amount of interferon injected and the natural killer cell activity was found; cytotoxicity peaked at 24 hours after injection of 3×10^6 units (a three-fold increase).

The exact mechanism by which interferon stimulates natural killer cell activity and by which natural killer cells lyse their targets is not fully understood. Some evidence suggests that interferon is able to induce differentiation of precursor cells into mature natural killer cells and to directly activate preexisting mature natural killer cells [99,100]. More recently, release of a natural killer cytotoxic factor has been shown in supernatants of natural killer cells exposed to appropriate tumor target cells, and this cytotoxic factor is believed to be involved in natural killer-mediated cytotoxicity [101]. Addition of alpha interferon to human lymphocytes results in augmentation of natural killer cytotoxic factor production [101]. Further in vitro studies suggest that alpha interferon may be required for both the production of natural killer cytotoxic factor and the modulation of its lytic activity [102].

The lysis of specific antibody-coated target cells, known generally as antibody-dependent cellular cytotoxicity, has also been shown to be mediated by large granular lymphocytes [103]. Several studies have indicated that both crude and purified alpha interferon preparations are able to augment antibody-dependent cellular cytotoxicity responses mediated by human lymphocytes in vitro [91,104]. This increase also occurred against target cells resistant to natural killer activity [105]. More recently, it has been shown that pure alpha interferon enhances the antibody-dependent cellular cytotoxicity of

human polymorphonuclear leukocytes against several hematologic cell lines in vitro [106]. Interestingly, the effect was most pronounced when the IgG antibodies in the antibody-dependent cellular cytotoxicity reaction were present in suboptimal amounts; this suggests that interferon may play a role in initial in vivo immune response when IgG levels are still low [106]. Interferon may augment this activity by increasing the expression of Fcγ receptors on the lymphocyte cell surface, enhancing the binding of immunoglobulin-coated target cells [107]. Like the natural killer cell response in human trials, antibody-dependent cellular cytotoxicity response in patients receiving pure alpha interferon is variable [108].

Monocyte Function. Monocytes and macrophages are bone marrow-derived cells that have the capacity of phagocytosis and pinocytosis and more recently have been shown to be tumoricidal in vitro and in animal models [109]. Like natural killer cell activation, alpha interferon in vitro enhances tumoricidal monocyte function [92,110]. Unlike natural killer cell activation, human clinical trials with both crude and recombinant alpha interferon have shown consistent activation of monocyte tumoricidal function [96,97]. The exact mechanism by which interferon activates monocytes in man remains unknown. Recent studies using recombinant interferon suggest that interferon acts as an inducer of macrophage Fc receptor-mediated phagocytosis [111]. Recombinant gamma interferon was significantly more potent than either alpha or beta interferon. Some studies suggest that gamma interferon is the major natural human lymphokine (known as the macrophage-activating factor) capable of inducing monocyte-macrophage tumoricidal activity [112]. The role of activated macrophages in tumor surveillance or tumoricidal activity in humans is currently under investigation.

B Lymphocytes. In vitro and in vivo studies of the effect of alpha interferon on immunoglobulin synthesis by B cells demonstrate that dosage and time of exposure are important. Pretreatment of human peripheral blood B lymphocytes with both crude and pure alpha interferon before addition of mitogen enhances immunoglobulin production, but interferon treatment after exposure to mitogen suppresses production [113,114]. Lower doses of alpha interferon enhance maturation of B cells, whereas suppression occurs at higher doses [115,116]. The enhanced immunoglobulin production occurs even when peripheral blood lymphocytes are separated into T and B cell subpopulations before interferon administration, suggesting a direct effect on B cells [113,114]. The first in vivo evidence suggesting an effect of interferon on antibody formation came from studies with mice [117]. Preliminary studies in human trials have demonstrated minimal increases of immunoglobulin secretion at 30×10^6 units, but not at other dosages [108]. Similar to the interferon effect on macrophages, gamma interferon has

been shown to be a more potent regulator of antibody response than alpha or beta interferon on an antiviral unit basis [118].

T Cells. T lymphocytes are the effector cells of cell-mediated immunity, and they perform a variety of functions including cellular cytotoxicity, helper and suppressor activity, and lymphokine production. The effect of interferon on T cells is complex: some phases of the T cell responses may be enhanced and others depressed. In vitro, alpha interferon enhances the cytotoxicity of the mixed lymphocyte cultures; however, proliferation is inhibited [119]. Both inhibition and activation of T suppressor cells from mixed lymphocyte cultures have been observed [120,121]. In clinical trials, both crude and recombinant interferon has been shown to depress lymphoproliferative response to mitogens and mixed lymphocyte culture [96,97]. The importance of interferon's effects on T cells as it relates to antitumor effect is not known.

Modulation of Cell Surface Antigens. Interferon induces a variety of changes in the cell surface including increases in the expression of Fc receptors on lymphocytes and macrophages that enhance tumoricidal activity [107,111]. Consistent, increased expression of HLA antigens A, B, and C and the HLA subunit B₂-microglobulin is observed with alpha, beta, and gamma interferon, both in vivo and in vitro [122,123]. Only gamma interferon has consistently increased expression of HLA-DR [124]; moreover, human gamma interferon, unlike alpha or beta, is able to increase expression of HLA-A, B, and C proteins on the cell surface at concentrations considerably lower than those necessary to induce an antiviral effect [125]. Because the HLA-DR system in humans appears to play a major role in the presentation of antigen for immune response [126], gamma interferon may have a more important role in treatment directed at cell surface proteins than either alpha or beta interferon.

Oncogene Expression. Neoplastic transformation of normal cells to malignant cells is now believed to be regulated by expression of cellular oncogenes. Rat fibroblast cells exposed to the Rous sarcoma virus undergo malignant transformation resulting from the expression of the viral *src* oncogene. The product of this gene has been shown to be a tyrosine phosphokinase (pp60^{src}) [127] that is capable of inducing this transformation. Treatment of Rous sarcoma virus-transformed rat cells with rat crude alpha interferon resulted in a 50 percent decrease in intracellular pp60^{src}-associated protein kinase activity and a more normal growth pattern [128]. Moreover tritiated leucine pulse labeling experiments showed that interferon worked by selectively reducing the synthesis of the *src* gene product [128].

Recombinant human alpha interferon has been shown to decrease accumulation of the cellular *myc* oncogene messenger RNA in the Daudi cell line (Burkitt's lymphoma) [129]. The effect is dose-dependent and occurs

before any inhibition of cell growth can be detected. Interestingly, no effect was seen on *c-myc* transcription rates, but rather an accelerated degradation of *c-myc* messenger RNA was noted (67 to 80 percent decrease in *c-myc* messenger RNA half-life) [129].

Effect of crude alpha interferon on oncogene expression in peripheral blood cells from two patients with chronic myelogenous leukemia has also been studied [130]. Although the expression of several oncogenes (*sis*, *ras*-Harvey, *ras*-Kirsten, and *myc*) remained unchanged during interferon therapy, a significant decrease in *abl* oncogene expression was detected within a few days after treatment was initiated in both patients. The results of these three studies suggest another mechanism by which interferon may inhibit tumor growth.

MECHANISMS OF INTERFERON ACTIVITY IN SPECIFIC DISEASES

Hairy cell leukemia is the model disease for studying the effects of alpha interferon. Patients with hairy cell leukemia have a severe deficiency in natural killer cell activity. Recovery of natural killer activity has been reported [24,131] in most patients with hairy cell leukemia after alpha interferon therapy. The recovery of natural killer cells paralleled hematologic recovery. It remains unclear whether the natural killer cells played a direct role in hematologic recovery or were simply a byproduct of interferon-induced hematologic recovery. However, it was of interest that the low natural killer activity in the untreated cells was not really attributable to a relative deficiency or dilution of the effector cells because the percent of Leu11-positive cells, which identify the natural killer cells, was within the normal range. This suggests that alpha interferon activated these cells into functional effector cells [24]. In addition to natural killer cell recovery, improvement in the total numbers of T lymphocytes including both helper and suppressor populations and monocytes paralleled the improvement in the other hematologic parameters after alpha interferon therapy.

Hairy cell leukemia and low-grade lymphomas are both indolent diseases of B cell origin. Alpha interferon has a high degree of activity in both diseases [18-27,29-35]. The lack of responsiveness of another indolent B cell malignancy, chronic lymphocytic leukemia, has as yet been unexplained [35,40-44]. A comparison of binding of iodinated recombinant alpha interferon to normal peripheral blood mononuclear cells, hairy cell leukemia cells, and chronic lymphocytic leukemia cells demonstrated that hairy cells bound approximately twice as much iodinated interferon as chronic lymphocytic leukemia and normal cells; however, the hairy cells had twice the surface area, which may explain the greater number of receptors [132].

Alpha interferon has been reported to induce cell surface and intracellular proteins in patients with hairy cell leukemia [133]. Autoradiographic analysis of one-dimen-

sional polyacrylamide gels showed induction of at least six proteins in nine patients treated with recombinant alpha interferon. Overall protein synthesis was not significantly altered. Some of these proteins were in the cell membrane, leading the investigators to suggest that interferon induces a protein signal in the hairy cell enabling their destruction [133].

Most recently Baldini and coworkers [134] isolated hairy cells from the spleens of previously untreated patients and cultured them in the presence of recombinant human alpha interferon. Monoclonal antibody surface marker studies revealed a significant enhancement of class II HLA antigen (HLA-DR). Because HLA antigens have been shown to be involved in cell-mediated cytotoxicity [126], they speculated that selective enhancement of class II HLA antigen may be another *in vivo* therapeutic mechanism of alpha interferon.

CONCLUSIONS

The importance of interferon as a direct antitumor agent or a biologic response-modifier remains an unanswered

question in the treatment of malignant diseases. Although it is clear that interferon will not be effective in most cancers, we have reviewed interferon's effectiveness in managing some of the hematologic malignancies. Even in these diseases, the optimal dose of interferon is uncertain. High doses may have greater direct antiproliferative activity, yet they may also suppress the immune system. Low doses may be more effective in enhancing the immune system. Interferon's role as a first-line treatment or in combination with standard cytotoxic drugs or other biologic response-modifiers is an area of ongoing research. Regardless of the eventual role of alpha interferon in the treatment of cancer, it is an important first member of a family of biologic response-modifiers used in treating human malignancies.

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