**Mechanism of Interferon Action in Hairy Cell Leukemia: A Model of Effective Cancer Biotherapy**

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Abstract

Hairy cell leukemia (HCL) is one of the few malignancies for which α-interferon (IFNa) is considered effective first-line therapy. However, the mechanisms of action of this agent in vivo have been the subject of much debate; in particular, the issue of whether clinical improvement in IFNa-treated HCL patients is dependent upon enhancement of host defenses or upon direct actions of IFNa upon the hairy cell remains unresolved. In this review, we examine the evidence supporting both lines of argument and synthesize this information within the framework of clinical studies of IFNa in HCL, the purpose being to determine which proposed mechanisms of IFNa action are indeed effective in vivo. From our analysis, it appears that the beneficial effects of IFNa upon immune function are important in decreasing the frequency of infectious complications of HCL but that these effects are probably not responsible for hairy cell elimination and cannot therefore account for major responses to IFNa therapy. We conclude that the primary mechanism of action of IFNa in HCL involves the induction of hairy cell differentiation towards a stage less responsive to growth factor stimulation, the primary consequence being proliferative inhibition. These effects may mimic events that occur during normal lymphocyte development, suggesting that the benefits of biotherapeutic agents might best be harnessed via studies of the effects of multiple and sequential biological response modifiers upon the growth and differentiation patterns of normal and malignant cells. Hairy cell leukemia could thus serve as an excellent model in which to investigate combined cancer biotherapy; the implications of our present understanding of IFNa in HCL to the biotherapy of cancer are discussed.

Introduction

The general approach to the study of cancer treatment has changed significantly in recent years. Whereas in the past emphasis was placed upon searching for better cytotoxic chemotherapeutic drugs, we now appreciate the fundamental vulnerabilities of such agents and have refocused much of our attention to more natural modalities. Research into the diverse effects of cytokines has been particularly fruitful, and we now possess greatly improved insight into the events involved in the regulation of immune response. The discovery that these same molecules may have direct effects upon neoplastic cells caused much excitement and gave rise to the concept of cancer biotherapy. Advances in recombinant DNA technology and the subsequent mass production of such agents rendered this idea practically feasible, and the 1980s marked the emergence of such factors in clinical trials, as lone agents, and in chemotherapeutic protocols. Agents thought to be most promising are IFNa, IL-2, and TNF. Although all three are presently involved in clinical trials, IFNa is the only cytokine to be approved by the Food and Drug Administration as first-line therapy for cancer (1).

α-Interferon has been tested in vitro and in vivo against a wide variety of malignancies, including common solid tumors, melanoma, sarcomas, lymphomas, and leukemias. In vitro studies and early trials were extremely encouraging, and IFNa was touted as a potential cure for many cancers. IFNa has shown some effectiveness in patients with metastatic melanoma (2), bladder carcinoma (3), renal cell carcinoma (4), multiple myeloma (5), some forms of non-Hodgkin's lymphoma (6), and CML (7, 8). However, clinical trials over the last 5 years have not yielded the spectacular results that were earlier anticipated, and the long-term prognosis for most IFNa-treated cancer patients remains essentially unchanged. As a result, one now perceives within the medical community a leveling in the enthusiasm for IFNa as an antineoplastic agent.

We feel that this is premature and that further studies are required to justify such pessimism. One critical problem is that although much is known about the biological effects and biochemical reactions associated with IFNa in vitro, little is understood about which of these events are the clinically significant mechanisms of action. Until this vital information is acquired, we will be unable to assess the full potential of interferon in cancer; it might be that adjunctive therapy could be designed to augment the specific actions of IFNa in certain cancers and thereby maximize its effectiveness.

HCL is one of the few malignancies for which IFNa has assumed a prominent therapeutic role (9). However, much controversy still exists concerning the mechanism of interferon action in HCL. Because the treatment of HCL with IFNa currently represents the best example of successful cancer biotherapy, increasing attention has been paid in recent literature to answering this question. The major goals of this review are: (a) to describe the effects of IFNa upon immune function in HCL patients; (b) to delineate the specific actions of IFNa upon the individual hairy cell and to determine whether a governing principle such as cellular differentiation can be invoked to explain them; and (c) to correlate this analysis with in vitro studies of IFNa action and to thus deduce which proposed mechanisms of action are actually involved in the clinical response to IFNa. Such knowledge could be utilized to provide further direction in IFNa research and to design methods of optimizing the effects of IFNa in HCL treatment. These principles could in turn be applied to the challenge of improving cytokine therapy in other malignancies.

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3 The abbreviations used are: IFNa, α-interferon; IL-2, interleukin 2; TNF, tumor necrosis factor; CML, chronic myelogenous leukemia; HCL, hairy cell leukemia; CLL, chronic lymphocytic leukemia; sIL-2R, soluble interleukin 2 receptor; IL-2R, interleukin 2 receptor; NK, natural killer; FACS-fluorescence-activated cell sorting; LAK, lymphokine-activated killer; BCGF, B-cell growth factor.
Hairy Cell Leukemia and Its Treatment with Interferon

Hairy cell leukemia is a chronic lymphoproliferative disorder that is characterized clinically by splenomegaly, pancytopenia, and the presence of a malignant clone of tartrate-resistant acid phosphatase-positive cells (10–12) displaying unique morphological features in the peripheral blood and bone marrow. The origin of the hairy cell has been the subject of much debate. Although evidence for monocytic origin has been presented (13, 14) and T-cell variants described (15), the bulk of the evidence favors a B-cell origin for these neoplastic cells. Hairy cells express surface immunoglobulin (16) and other B-cell-associated surface antigens (17), and molecular biological studies have revealed the presence of immunoglobulin gene rearrangements in these cells (18). Studies with monoclonal antibodies and differentiation inducers indicate that hairy cells probably stem from preplasmatic B-cells, and HCL is therefore classified as a B-cell malignancy slightly more differentiated than CLL (19, 20). HCL, like CLL, runs a chronic course, with median survival being over 5 years from the time of diagnosis prior to the introduction of systemic therapy (21).

Although treatment options for HCL have included chemotherapy, radiation, leukapheresis, androgens, and bone marrow transplantation (22), at present the primary recommended therapies are splenectomy, IFNα, and pentostatin (in clinical trials). Splenectomy results in the alleviation of symptomatic splenomegaly, and approximately 50% of patients undergoing splenectomy achieve normalization of peripheral blood counts (22). However, splenectomy has no effect upon bone marrow disease, and most patients eventually require additional therapy; hematological parameters and the degree of bone marrow cellularity are now utilized to identify subsets of patients who might derive long-term benefit from the procedure (23). The various systemic treatment modalities used thus far have had serious limitations and have generally failed to produce durable responses. Clinical trials involving pentostatin have demonstrated overall response rates of greater than 95%, of which 75% have been complete responses (24–26). However, the long-term toxicities of this agent have not yet been determined, and in particular, reports of delayed immunosuppression in HCL patients receiving pentostatin raise concerns that must be resolved before this drug can be used as first-line therapy in HCL patients (27). Recently, durable complete responses with minimal toxicity were reported to occur in 11 of 12 HCL patients treated with 2-chlorodeoxyadenosine (28). These results appear to be quite promising, although the safety and efficacy of this drug in HCL must be further evaluated in a larger series of patients.

At present, IFNα represents the primary systemic therapy for patients with HCL. Most HCL patients treated with IFNα show a favorable clinical response to the drug, overall response rates being approximately 80–90%, with a 5% complete response rate (21). It has now been established that the clinical response to IFNα differs from that generally seen with chemotherapeutic agents. A prompt response to standard chemotherapy is usually seen if treatment has been effective. In contrast, the response to IFNα may develop slowly, and even partial responses may be quite durable with continuing treatment. Recovery of platelet count can occur within 2 weeks, and reduction of splenic and peripheral blood tumor burden generally occurs within 2 months. Neutropenia resolves in 2–3 months, and erythrocyte and neutrophil counts may continue to increase for up to 12 months (21, 22). Bone marrow improvement generally occurs over a period of 2–6 months, although eradication of the disease does not seem to occur, and relapse occurs in most patients after discontinuation of IFNα. Current recommendations are for 12 months of therapy (21); the median time to relapse after discontinuation of IFNα is approximately 2 years (29). IFNα is successful in reinducing remission in most of these patients. These results suggest that despite its low complete response rate, IFNα therapy may lead to lasting clinical remissions more consistently than have previous forms of treatment. In addition, IFNα, a biological agent, exhibits a side effect profile which compares very favorably with those of standard chemotherapeutic agents, including pentostatin. Unlike pentostatin, IFNα can be utilized safely in patients with active infections. Consequently, IFNα is presently the treatment of choice for most HCL patients in need of systemic therapy (21).

Two general mechanisms have been proposed to explain the ability of IFNα to induce remissions in HCL patients: enhancement of host immune responses; and direct antiproliferative effects upon the hairy cell.

Effects of Interferon upon Immune Function

α-Interferon has been demonstrated to exert effects at multiple levels in the generation of immune response: it influences the production and differentiation of hematopoietic elements; enhances their recognition of foreign antigens; and augments the ability of effector cells to destroy their selected targets. The relevance of such actions to the biotherapy of HCL has been the subject of intense debate. In particular, the issue of whether IFNα-enhanced immune function mediates hairy cell elimination is central to our understanding of the actions of IFNα in HCL.

Effects upon the Hematological Profile. One of the primary clinical manifestations of HCL is the marked pancytopenia that often afflicts its victims, predisposing them to symptomatic anemia, bleeding complications, and recurrent infections. In particular, the profound degree of monocytopenia found in HCL patients has long been recognized as a prominent feature of the disease (30). Severe impairments in natural killer cell activity have been noted (31), and defects in T-lymphocyte immunity have also been reported, rendering HCL patients more susceptible to infections with atypical organisms as well as common bacterial pathogens (32). Infectious complications represent the leading cause of death in patients with HCL (21). It has long been believed that the cytopenias observed in HCL are the result of splenic sequestration of hematopoietic elements and bone marrow infiltration by hairy cells. Indeed, splenectomy has been shown to result in improvement in at least one hematological parameter in 90% of cases (21). However, the alleviation of pancytopenia is often incomplete, and even patients responding well to splenectomy eventually experience recurrence of cytopenias. It has therefore been suggested that the pancytopenia in HCL is due primarily to impaired production of hematopoietic elements rather than to hypersplenism (12). This may in part be due to space-occupying bone marrow infiltration by leukemic cells; however, recent evidence favors a role for hairy cell-derived substances that inhibit the growth and differentiation of specific hematopoietic cell types.

Most investigations of such phenomena have focused upon the presence of sIL-2R in the sera of patients with HCL. It has long been known that the malignant cells in most HCL cases express the CD25 (Tac) antigen (18), which is thought to represent a determinant on the interleukin 2 receptor. This
molecule is normally expressed on activated T-lymphocytes and on a subset of activated B-lymphocytes (33); in these cell types, the IL-2R mediates responsiveness to IL-2 and thus plays an important role in cellular proliferation.

In 1985, the release of a soluble form of the IL-2R from activated human lymphocytes in vitro was first reported (34). Soon afterwards, the sera of eight untreated HCL patients were tested, and it was discovered that all eight samples contained high levels of the sIL-2R (35). Other investigators soon confirmed these findings in larger series of patients (36, 37). It was therefore suggested that the sIL-2R might play a role in the impairment of T-lymphocyte and natural killer immunity observed in HCL patients by competitively inhibiting the binding of IL-2 to its receptors on T- and NK cells, depriving them of an important activation/proliferation stimulus. In addition to their role in preventing infections, T-lymphocytes and NK cells produce a multitude of cytokine growth factors that serve to regulate bone marrow stem cell function. Hence, a paucity of these elements might disrupt critical balances in the normal hematopoietic environment and further contribute to pancytopenia.

Various leukemia-associated bone marrow inhibitors have been characterized over the last 15 years (38, 39), but until recently, attempts to isolate hairy cell-derived factors that might mediate hematopoietic stem cell suppression had not met with success (40). During the last few years, several groups have isolated activities from the sera of HCL patients that inhibit in vitro colony formation by bone marrow stem cells. Two groups reported on factors the inhibitory activity of which appeared to be primarily directed against myeloid and erythroid progenitor cells (41, 42). One was shown to be a protein of M, 5000–6000 the other is presently awaiting molecular characterization. In addition, it was recently reported that bone marrow sera from HCL patients contain a tumor necrosis factor that causes significant progenitor cell suppression in colony-forming assays (43). Although further characterization of these factors is necessary, their presence might explain the defect in monocytic differentiation seen in HCL patients. Adverse consequences associated with monocytopenia may include reductions in phagocytic activity and antigen-presenting ability, along with decreased synthesis of important monokines such as interleukin 1 and IFN-α. Deficiencies in these compounds might cause further suppression of multiple cell lineages.

The beneficial effects of low-dose IFN-α upon the hematological profile of patients with HCL have been firmly established (22). Patients responding to IFN-α with significantly diminished hairy cell burden often begin to experience alleviation of cytopenias before hairy cell percentages in the bone marrow start to decrease (44). Furthermore, it has been demonstrated that even patients not achieving complete or partial bone marrow responses display significant improvement in bone marrow function, as is evidenced by decreased infection rates and blood product transfusion requirements (45). It is thus clear that the effects of IFN-α upon peripheral counts occur partly via pathways that do not depend upon reduction of tumor load and fibrosis.

α-Interferon is known to exert effects upon multiple cell types involved in normal immune response. In particular, it has been shown to promote cellular activation and differentiation in cells of several hematopoietic lineages, including monocytes (46). Because of the profound monocytopenia seen in HCL, it has been postulated that a state of endogenous IFN-α deficiency exists in these patients. Indeed, IFN-α biotherapy in HCL has been likened to insulin "replacement" therapy in diabetes mellitus (47), this theory holding that exogenous IFN-α may initially compensate for the lack of endogenous IFN-α, resulting in resumption of normal IFN-α-dependent hematopoietic processes, such as monocytic differentiation. These effects might occur quite early in the course of therapy and may in part explain why HCL patients experience clinical improvement prior to reduction in the hairy cell index.

Decreased helper/suppressor T-cell ratios have been noted in HCL patients (48), consistent with observed deficiencies in cell-mediated immunity. Elevated levels of soluble CD8 have been discovered in the sera of these patients (49); this finding is thought in part to signify increased suppressor cell activity. IFN-α has been shown to drastically reduce soluble CD8 levels as well as the number of CD8+ T-cells in the peripheral blood. It has been shown that this reduction, with the resultant restoration of normal helper/suppressor ratios (50), correlates well with the alleviation of bone marrow suppression seen in IFN-α-treated HCL patients (49).

A third route by which IFN-α improves peripheral blood parameters may involve interruption of humoral pathways of hairy cell-mediated bone marrow suppression. One group reported that the sera of patients responding well to IFN-α contained significantly less stem cell-inhibitory activity than those of untreated patients (42). These reductions correlated well with decreases in peripheral blood hairy cell counts and may therefore have reflected changes in tumor load and factor-secreting capacity. The issue of whether IFN-α causes changes in the production of this inhibitor before reductions in hairy cell index are seen is at present unclear. Studies concerning the function of other hairy cell-derived factors during IFN-α therapy have not yet emerged.

A fourth major mechanism by which IFN-α may improve immune function in HCL patients revolves around its ability to decrease serum levels of soluble interleukin 2 receptor. In the original study on eight HCL patients with high sIL-2R levels, it was reported that IFN-α treatment resulted in steadily decreasing serum sIL-2R levels throughout the 12-month course of therapy in all patients tested. A strong correlation between sIL-2R levels and clinical status was seen, and a possible role for this parameter in monitoring response to IFN-α therapy was suggested (35). Other groups have since confirmed and extended these observations (36). In studies at our institution (37), a statistically significant correlation between sIL-2R levels and hairy cell index was found, demonstrating that much of the reduction in sIL-2R level may be due to the decrease in tumor load seen with IFN-α treatment. Diminished levels of sIL-2R may result in exposure of sensitized cells to increased levels of available IL-2 and may thus enhance their ability to clonally proliferate in response to antigenic challenge. Further studies are required to determine if levels of sIL-2R can be correlated with susceptibility of HCL patients to typical and atypical infections.

At present, the mechanisms underlying the hematological improvement seen in IFN-α-treated HCL patients have not yet been fully elucidated. However, it is clear that these actions encompass alterations in the production of a wide range of cell types, including monocytes, neutrophils, erythrocytes, platelets, and various lymphocyte subsets. Such changes may result in restoration of essential humoral and cellular balances, affording the body a more appropriate hematopoietic milieu in which to continue synthesis of important immune elements. IFN-α may also improve immune response by increasing the availability of...
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cytokines essential for antigen-dependent cellular proliferation. Significant reductions in infection frequency attest to the clinical significance of these effects.

Effects upon Antigen Recognition. Recognition of foreign antigens by the human immune system is mediated by products of the major histocompatibility complex (HLA gene locus). Class I antigens, expressed in cells from most human tissues, are thought to target malignant and virus-infected cells for destruction by CD8+ cytotoxic T-lymphocytes. Class II antigens, limited mainly to B-lymphocytes, monocytes, and macrophages, are involved in the presentation of foreign or autoantigens to CD4+ helper T-cells, and the subsequent development of antigen-specific clones of T-lymphocytes and antibody-producing B-lymphocytes (51). HLA antigens are expressed upon malignant cells in a wide variety of human and animal neoplasms; it is hypothesized that they may play an important role in the recognition of tumor cells by the immune system (52, 53). Malignant cells from most HCL patients express HLA-ABC (Class I) and HLA-DR (Class II) antigens, consistent with the B-cell origin of these cells (17).

α-Interferon has been demonstrated to modulate expression of HLA determinants on many normal and malignant cell types. It has been shown to enhance Class I determinant expression on B-lymphocytes (54), on monocytes, and on tumor cells from various malignancies, including Burkitt's lymphoma and CML (55). Class II determinant expression on B-lymphocytes and monocytes is generally not affected by IFNα (54); however, IFNα has been shown to induce and modulate Class II antigen expression on neoplastic cells from CML (55) and metastatic melanoma (56), cancers in which IFNα has demonstrated some therapeutic value.

In 1986, FACS analysis was used to show that hairy cells cultured with IFNα demonstrate significantly increased reactivity with the OKDR, Leu-10, and OKIa monoclonal antibodies (57). Further studies utilizing cytofluorography and immunoelectron microscopy confirmed these findings and reported that the observed enhancements in HLA Class II expression occur in HCL patients responding to IFNα therapy but not in non-responders or in control cells from normal individuals and patients with other B-type leukemias (58–60). However, it has not been shown that this is a primary cause of favorable responses to IFNα therapy; the finding of increased HLA expression may merely indicate that IFNα exerts direct effects upon the hairy cells of responders and that increased HLA expression is a marker of hairy cell sensitivity to antiproliferative effects of IFNα. Further studies are required to clarify the issue of whether changes in Class II HLA antigen expression render hairy cells more susceptible to recognition by immune elements.

Another question which remains to be answered involves the expression of Class I HLA antigens upon normal and malignant cells in HCL patients. IFNα has been reported to cause increased expression of Class I determinants on hairy cells (60). However, this finding may be of little functional significance, inasmuch as it has been repeatedly demonstrated that hairy cells are poor targets for major histocompatibility complex-restricted cytotoxic T-cell lysis and that this mechanism is probably not an important feature of the immune defense against HCL (61, 62). Nevertheless, the ability of IFNα to induce Class I HLA antigens on normal B-lymphocytes and monocytes in HCL patients may result in improvements in the antigen-presenting ability of monocytes or in increased sensitivity of B-cells to T-cell-derived signals. This may serve as another mechanism by which IFNα decreases the frequency of infectious complications in HCL patients.

Effects upon Effector Cell Cytotoxicity. NK cells have long been considered likely mediators of antitumor activity (63). They are thought to function as important elements in the nonspecific defense system, accomplishing non-major histocompatibility complex-dependent lysis of abnormal or neoplastic cells. Severely deficient NK cell function has been widely reported in HCL patients (64, 65); it was thought that this impairment might contribute to disease progression and an increased predisposition to infections. Mechanisms underlying this deficit may include decreased production of NK cell precursors due to HCL-induced bone marrow suppression, deficient activation of such cells due to soluble IL-2R-mediated reductions in available IL-2, target binding defects (65), and impairment of cytolytic mechanisms.

α-Interferon has been shown to augment NK cell cytotoxicity against a variety of neoplastic cells, including those from several hematological malignancies (66, 67). IFNα exerts beneficial effects upon the NK system of HCL patients; whether these actions are essential to the elimination of hairy cells has been an issue of controversy. IFNα has been reported to increase the number of large granular lymphocytes, precursors of NK cells, in the peripheral blood of HCL patients (64), although conflicting reports exist concerning changes in peripheral blood NK cells (49, 68). Studies have clearly demonstrated significant increases in the cytolytic capabilities of NK cells against K562 tumor cell targets after in vivo IFNα treatment of HCL (64, 69). One group recently reported improved NK-K562 cell binding, increased killing ability of bound targets, and increased release of natural killer cytotoxic factor by NK cells preincubated with IFNα (70). Several studies have found correlations between improvement of NK function, hematological improvement, and decreases in sIL-2R levels and hairy cell index in IFNα-treated patients (60, 69, 71, 72). However, improvements in NK function tend to occur late in the course of IFNα therapy and have not been shown to precede hematological recovery and tumor load reduction (69, 72).

The most compelling evidence speaking against the role of NK cells in hairy cell killing comes from studies which have clearly demonstrated that hairy cells are not susceptible to lysis by NK cells despite induction with IFNα (64, 73, 74). Lack of hairy cell recognition and binding by NK cells has been implicated as a cause for this resistance (61, 65). Hairy cells are also resistant to lysis by IFNα-preincubated LAK cells (75). It is therefore highly unlikely that NK/LAK-mediated cytolysis plays an important role in the elimination of malignant cells in HCL. However, IFNα-boosted enhancement of NK/LAK cytotoxicity may improve the deficient immune function observed in HCL patients and may thus contribute to the reduced frequency of opportunistic infections that is seen after IFNα therapy.

Summary: Interferon and Immune Function in Hairy Cell Leukemia. The effects of IFNα upon the immune system in patients with HCL are quite complex and involve actions at multiple levels of immune function: production of hematopoietic elements; recognition of foreign antigens; and killing of selected targets. One issue critical to our understanding of the mechanisms of action of IFNα in HCL concerns the extent to which changes in hairy cell index can be explained by IFNα-induced augmentation of host immune function. Our review of the literature leads us to conclude that the existing evidence does not support a major role for IFNα-mediated enhancement of immune function in the elimination of hairy cells and that...
these actions cannot account for the dramatic responses to IFNα therapy seen in HCL patients. Because IFNα does not completely eradicate hairy cells from the bone marrow, the major goal of therapy is not necessarily to cure the disease but rather to prolong quality survival, in part by reducing the frequency and severity of HCL-associated complications. Improved host defenses in IFNα-treated HCL patients do appear to prevent opportunistic infections, the leading mortality-asssociated complication of HCL; these effects may be critical during the early stages of therapy, in which the full effects of IFNα upon the hairy cell index have not yet occurred. Hence, IFNα-induced improvements in immune function play an important role in the treatment of HCL.

Direct Effects of Interferon upon the Hairy Cell

HCL patients responding well to IFNα therapy demonstrate dramatic reductions in hairy cell burden in both the peripheral blood and bone marrow (21). Because existing evidence does not support the possibility of significant hairy cell elimination by IFNα-enhanced host defenses, an explanation for the observed improvement may be rooted in direct effects of IFNα upon the hairy cell. IFNs have been shown to inhibit the proliferation of various normal and malignant cell types (76, 77) and have even been associated with phenotypic reversion of transformed cells (78, 79). The delayed timing of IFNα-induced cytoreduction in HCL, the paucity of complete responses to IFNα therapy, and the apparent failure of IFNα to completely eradicate hairy cells from the bone marrow serve to dismiss the idea of IFNα as a cytotoxic agent in HCL. Rather, it is thought that IFNα functions as a cytostatic agent in HCL, inhibiting hairy cell proliferation and thereby accomplishing decreases in tumor burden.

Although experiments in recent years have enabled us to describe in some detail the diverse effects of IFNα upon the hairy cell, many questions arise regarding the relevance of these independent actions to hairy cell function and to the clinical improvement seen in HCL patients. Can these changes be understood in the context of normal B-lymphocyte development? Why is IFNα so effective in HCL but not in CLL and other related lymphoproliferative disorders? We support the view that IFNα-induced hairy cell differentiation accounts for most of the observed changes in hairy cells exposed to IFNα and for the clinical efficacy of this agent in HCL. The characteristics of IFNα-treated hairy cells include a more mature phenotype and an impaired response to growth factors. The major consequence is a reduction in proliferative capacity.

Effects upon Response to Growth Factors

Leukemic cells are believed to exhibit phenotypes which correspond to discrete stages in hematopoietic differentiation. It has therefore been postulated that these cells may respond to the same cytokine growth factors as their nonmalignant counterparts. Experiments utilizing differentiation inducers in conjunction with monoclonal antibodies have demonstrated that hairy cells probably represent malignant partially activated B-lymphocytes, slightly more mature than the neoplastic “resting” B-cells of chronic lymphocytic leukemia (19, 20). Normal B-cells at this level of maturity are beginning the process of clonal proliferation and eventual differentiation into antibody-secreting plasma cells; hence, they are quite responsive to a variety of biological response modifiers. It has therefore been proposed that these same molecules might control the proliferation of hairy cells in vivo.

B Cell Growth Factor. Hairy cells in vitro and in vivo demonstrate a low proliferative index in comparison to other malignant cells, even when stimulated with standard B-cell mitogens, and have thus been difficult to maintain in long-term culture. However, the addition of BCGF to cultured hairy cells has been shown to stimulate hairy cell growth (80), as illustrated by increased thymidine incorporation and by the maintenance of continuous hairy cell lines. As a result, it was suggested that BCGF might contribute to hairy cell proliferation in vivo. In 1987, cytotoxic extracts from hairy cells of untreated patients were found to contain a BCGF-like activity when tested on hairy cells as well as on normal BCGF-dependent human B-cell lines. Chromatographic analysis indicated that this activity was similar to the activity of normal T-cell-derived cytosolic BCGF. This study suggested that hairy cells contain, and in some cases secrete, an autostimulatory growth factor, implicating autocrine growth mechanisms in the pathophysiology of HCL (81).

In 1986, it was first demonstrated that IFNα inhibits BCGF-induced hairy cell growth using six cultured cell lines (82). This finding was quickly confirmed by other investigators, who observed similar phenomena in hairy cells taken from IFNα-treated patients (83). These studies were extremely important, because they represented the first proofs of the direct antiproliferative actions of IFNα on hairy cells. Taken in conjunction with the report of HCL-derived BCGF, they suggested that the mechanism of IFNα action in HCL may involve inhibition of autocrine loops that serve to promote hairy cell proliferation.

Interleukin 2. The expression of the IL-2 receptor on subsets of activated B-cells was a source of confusion until it was shown that IL-2 may play a direct role in B-cell proliferation and differentiation (84, 85). The presence of the CD25 (Tac) antigen on hairy cells supports the concept that hairy cells are neoplastic counterparts of CD25+ partially activated B-cells. IL-2 alone has been shown to exert only minor proliferative effects upon hairy cells and does not appear to possess the ability to maintain hairy cells in culture (80). However, it has been demonstrated that treatment of activated B-lymphocytes with BCGF greatly enhances the surface expression of IL-2R (86); immunoelectron microscopic studies in our laboratory have extended these observations to include hairy cells, revealing significant enhancement of CD25 expression in BCGF-treated hairy cells (87). These findings are compatible with the notion that BCGF recruits normal and malignant B-cells to become responsive to IL-2 by increasing their surface expression of the IL-2 receptor.

As discussed earlier, the sera of patients with HCL have been found to contain elevated levels of soluble IL-2R, and IFNα has been shown to greatly reduce these levels (35). Studies have since demonstrated strong correlations between IFNα-induced sIL-2R reduction and clinical response to therapy (36). A correlation between serum sIL-2R level and hairy cell index has been noted (37); however, it is not clear to what extent reductions in sIL-2R simply reflect decreases in tumor burden, rather than decreased expression of IL-2R on hairy cells. In 1989, FACS analysis was used to show decreased CD25 expression on hairy cells cultured with IFNα (62). Our immunolultrastructural studies have confirmed these observations via examination of individual hairy cells exhibiting persistently diminished expression of CD25 after 3 days or more of culture with IFNα (87). In addition, IFNα consistently abrogated BCGF-induced increases in CD25 expression on hairy cells in these experi-
ments. It is therefore likely that part of the action of IFNα in HCL involves decreasing surface IL-2R expression, rendering hairy cells immune to the proliferative effects of IL-2.

The strong correlations found between decreases in sIL-2R level and clinical response to IFNα therapy reflect several ongoing processes, one of which is probably tumor load reduction. However, it is now quite clear that IFNα also causes decreases in IL-2R expression on individual hairy cells. On the one hand, this reduction may simply reflect alterations in hairy cell function due to antiproliferative effects of IFNα, unrelated to IL-2. Alternatively, decreases in responsiveness to IL-2 may in part mediate the antiproliferative effects of IFNα on hairy cells. We believe that BCGF-treated hairy cells represent a unique IL-2-sensitive stage of B-cell development, that promotion of further differentiation by IFNα results in diminished sensitivity to BCGF and IL-2, and that these changes are in large part responsible for the clinical response to IFNα.

Tumor Necrosis Factor. TNF is a monocyte-derived cytokine that mediates diverse effects as a modulator of nonspecific immune response (88). Although clinical investigations of TNF have focused upon its role as a cytotoxic agent against various human cancers (89), it has also been demonstrated to stimulate the proliferation of several normal and malignant cell types (90, 91). Hairy cells have been reported to express surface receptors for TNF (92), and treatment of activated hairy cells with TNF has been shown to elicit a proliferative response (93). In addition, hairy cells from some patients are thought to secrete TNF; evidence for this is derived from analysis of HCL culture supernatants and from the discovery of TNF in the bone marrow sera of untreated HCL patients (94). Treatment of hairy cells with TNF results in greatly enhanced cytosolic interleukin 1, interleukin 6, and TNF mRNA and protein levels (95). Hence, the TNF system clearly fulfills the criteria for an autocrine loop.

α-Interferon has been shown to inhibit TNF-dependent hairy cell growth in experiments by several investigators (93, 95). This effect appears to be independent of changes in surface TNF receptors. Following IFNα administration to cultured hairy cells, increased levels of cytosolic 2-5A synthetase mRNA are noted; soon afterward, decreases in mRNA levels for the above cytokines are seen (95). It has therefore been suggested that IFNα might alter hairy cell proliferation via degradation of cytokine mRNA. In view of the demonstrated TNF autocrine loop inhibition by IFNα in vitro, it is quite possible that diminution of TNF production by IFNα plays a role in mediating the antiproliferative effects of IFNα in vivo.

The above discussion illustrates the importance of B-cell growth-promoting agents in the pathophysiology of HCL. Other factors, including interleukin 4, interleukin 5, and interleukin 6, have been reported to alter hairy cell growth capabilities (93, 96), but these interactions have not yet been well characterized. Further studies are required to determine the extent to which hairy cell growth is dependent upon each of the above factors and to fully assess the consequences of inhibition by IFNα. Such experiments will need to control for the possible presence of multiple cytokines in the supernatants of hairy cell cultures. The discovery that IFNα may operate via the inhibition of autocrine and paracrine growth factor loops is indeed fascinating and suggests that further exploration into growth factor-stimulated proliferation of other tumor cell types may be necessary for cancer biotherapy to succeed.

Effects upon Oncogene Expression

Aberrant protooncogene expression is thought to contribute to the expression of transformed phenotypes in animal and human malignancies. Normal and malignant hematopoietic cells have often been used in investigations into these phenomena; as a result, much is known about oncogenic function in leukemic cells. In particular, the expression of the c-myc and c-fos genes has been intensively studied in the neoplastic cells of lymphoproliferative disorders. As a result, much has been learned about the nuclear regulation of lymphocyte proliferation, activation, and differentiation.

Cell cycle-dependent transient expression of the c-myc and c-fos genes has been shown to occur during the activation of human lymphocytes (97, 98). These genes encode nuclear proteins that are thought to be necessary for commitment to DNA replication and cell division in response to growth factors. It has been suggested that these proteins may activate cells to progress from a quiescent phase of the cell cycle (G0) into G1 (97) and thus accelerate their transition into S phase. Expression of either gene throughout the cell cycle might result in a loss of proliferative control due to an inability of the cells to remain in G0.

It has been demonstrated that levels of c-myc mRNA fall dramatically following the induction of differentiation of various cell types (99, 100). In 1984, it was found that treatment of various neoplastic lymphoid cells with type I interferons resulted in G0/G1 arrest in 10 of 11 cases in which proliferation was inhibited (101). In 1986, endogenous IFNα-induced c-myc suppression was shown to be a normal event during the differentiation of hematopoietic cells, and this reduction was seen to correlate with cellular arrest at the G0/G1 transition into the cell cycle (102). Selective reductions in c-myc mRNA in IFNα-treated Daudi-Burkitt's lymphoma cells were reported, and it was suggested that this might play a role in the inhibition of proliferation (103). These observations were soon confirmed and extended to include IFNα (104). Other studies have since shown that IFNα induces terminal differentiation of leukemic cells in part by reducing the half-life of c-myc mRNA (105, 106). Hence, IFNα is thought to play a major role in modulating protooncogene expression in normal and malignant lymphoid cells, the consequences of which include regulation of cellular proliferation and differentiation.

In 1986, the expression of the c-fos and c-myc genes in cells taken from HCL patients before and during IFNα therapy was examined (107). c-fos mRNA was detected in the hairy cells from nine of nine untreated patients, and IFNα was shown to modulate c-fos expression in both responding and nonresponding patients. However, c-myc transcripts were not detected in typical HCL cells but were present in both variant (nonresponding) cases tested. In these cases, IFNα failed to modulate c-myc expression even after 6 weeks of administration. The lack of expression of c-myc in typical HCL cases is consistent with the low proliferative index of hairy cells. The fact that IFNα successfully modulated c-fos production in these cases may indicate that IFNα does affect hairy cell function at the level of oncogene expression. It may be that this regulation is sufficient to inhibit proliferation in typical HCL cases but that c-myc expression in variant cases is capable of overriding the c-fos system, resulting in continued proliferation and a clinically resistant phenotype. The authors of this paper suggested that the failure to modulate c-myc expression might indicate the limits of low-dose IFNα therapy for HCL. In resistant patients, the failure of IFNα to
reduce c-myc levels might prevent the G0/G1 arrest and subsequent differentiation seen in other IFNα-treated cell types. Further studies are needed to delineate the cellular effects associated with c-fos expression in hairy cells and the relationship between the modulation of this gene by IFNα and growth factor-dependent control of the cell cycle.

Effects upon Hairy Cell Differentiation

The impressive responses of HCL patients to low doses of an apparently cytostatic agent compel one to consider mechanisms of action that mimic the physiological effects of IFNα upon normal cellular function. It has been proposed that the primary effect of IFNα in HCL is to induce hairy cells to differentiate into mature cells less responsive to proliferative signals. The rationale behind this theory is derived from the demonstration of the ability of IFNα to induce differentiation in a variety of normal and malignant cells, including leukemic cells of both lymphoid and myeloid origin (108–110). Evidence supporting the role of IFNα as a differentiation agent in HCL comes from numerous immunological and ultrastructural studies which clearly demonstrate phenotypic alterations in hairy cells treated with IFNα.

Most investigations of hairy cell differentiation have utilized monoclonal antibodies in conjunction with standard immunofluorescence methods or FACS to analyze surface expression of B-lymphocyte antigens. Early studies using these techniques led many researchers to conclude that IFNα-induced alterations in hairy cells are not accompanied by phenotypic maturation. However, in most of these experiments, only percentages of positive cells were examined; recent FACS data using modal fluorescence in combination with percentage positivity have reported different results, illustrating that percentage positivity and measurements of fluorescence per cell are not necessarily concordant. Methodologies such as modal FACS and immunoscanning electron microscopy are thus ideally suited to such investigations and may provide more accurate information regarding antigenic regulation at the single cell level.

A second problem lay in the interpretation of the results of early immunological studies. Because of the designation of HCL as a B-type lymphoproliferative disorder, assessments of hairy cell differentiation were often based upon measurements of surface and cytoplasmic immunoglobulin and plasma cell markers. However, it has since been shown that although hairy cells are mature B-cells that often express the plasma cell marker PCA-1(111), their patterns of differentiation differ significantly from those of normal B-lymphocytes. Treatment of hairy cells with phorbol esters to induce differentiation in vitro has been demonstrated to endow them with macrophage-like qualities; they adhere to plastic dishes and acquire ultrastructural properties similar to those of macrophages (112–115). The explanation for this strange transformation is at present unknown; it may be that the underlying pathophysiology of HCL involves an irreversible departure from the classical B cell differentiation program or that hairy cells require specific maturation factors in order to display plasmacytic features (116). Whether such factors capable of restoring typical B cell differentiation tendencies to hairy cells indeed exist is presently unknown. In addition, it has not been shown that terminal hairy cell differentiation is required for growth suppression to occur; hence, the inability of IFNα to cause terminal differentiation as measured by changes in immunoglobulin production and plasma cell antigen expression does not exclude partial hairy cell differentiation as a cause of proliferative inhibition. Like B-cells, maturing hairy cells lose positivity for many B-cell and hairy cell surface antigens as they progress. As a result, recent studies utilizing immunological methods to examine hairy cell development have been extremely valuable in characterizing IFNα as a differentiation stimulus for hairy cells.

Class II HLA antigens are expressed differentially during B-cell development and are thus regarded as differentiation markers in normal and leukemic B-lymphocytes (117). In particular, expression of these antigens and the CD25 (Tac) antigen has been associated with the activation of normal B-lymphocytes (33). The ability of phorbol esters to induce Tac expression on B-type CLL cells (118), to significantly enhance the expression of Class II HLA on CLL cells (119), and to provide them with the classical surface morphology of hairy cells (118) supports the reliability of these surface markers as indicators of malignant B-cell activation and differentiation.

Experiments in various laboratories have clearly shown that IFNα enhances the expression of Class II HLA antigens on hairy cells. In 1986, increased HLA-DR expression was reported to occur after incubation of samples from three HCL patients with IFNα in vitro (57). These findings were confirmed by others using various methods to compare antigenic expression in HCL patients who responded favorably to IFNα with patients refractory to therapy. In one study, leukemic cells exhibited a definite increase in HLA density and a slight decrease in transferrin receptors in responding patients but not in refractory patients. The authors suggested that the enhanced HLA expression indicated progression towards differentiation and that the decrease in transferrin receptors was correlated with growth reduction (58). In 1988, IFNα-induced increases in HLA-DR expression on hairy cells were shown to be related to favorable clinical course (60). More recently, changes in hairy cell surface immunoglobulin type without production of cytoplasmic immunoglobulin or increased expression of plasma cell markers were demonstrated to occur following IFNα therapy (116). It was suggested that these changes were consistent with the induction of partial differentiation but that further cofactors might be required to completely overcome the maturation arrest seen in the malignant hairy cells.

In 1989, a quantitative FACS study was performed upon splenic and peripheral blood cells taken from six HCL patients. A large panel of monoclonal antibodies was used, and both percentage positivity and modal fluorescence were utilized to analyze phenotypic alterations in hairy cells exposed in vitro to IFNα. The results agreed with those of previous investigators, demonstrating significantly enhanced HLA antigen expression on IFNα-treated hairy cells. Enhanced CD22 expression along with decreased staining for CD25, FMC7, surface immunoglobulin light chains, CD19, CD9, and HC2 were also found. It was proposed that the enhancement of HLA Class II and CD22 expression and the reductions observed in expression of the other antigens might be explained by activation/partial differentiation of the hairy cells, because these antigens tend to be lost during the later stages of B-cell differentiation. Treatment of hairy cells with phorbol esters resulted in similar antigenic changes, supporting this interpretation of the data. However, the IFNα-treated hairy cells did not become completely negative for CD19, CD22, and HLA Class II; this was taken to indicate a failure of the hairy cells to complete differentiation (62).

Our own studies involving the immunoscanning electron microscopy analysis of antigenic and ultrastructural changes on
IFNα-exposed hairy cells have allowed us to confirm that the conclusions reached by previous investigators are indeed valid at the level of the single hairy cell. We observed significantly increased HLA-DR expression and decreased CD25 expression on hairy cells cultured for 3 days with IFNα and further noticed a significant decrease in CD11c expression after 5 days of culture with IFNα (87). In addition, we described specific ultrastructural alterations in hairy cells treated with IFNα. Up to one-third of the cells displayed significant membrane changes including the appearance of a bud-like formation at one pole and a “bubbling” membrane covering the cell body and/or the surface ruffles. Redistribution of antigens upon the cell surface was also noted. These morphological effects were observed only upon cells displaying increases in class II HLA expression and decreases in CD25 expression after 3 days of culture with IFNα. The HCL patients from whom these cells were taken responded well to IFNα therapy in vivo. Normal B-cells, CLL cells, Daudi-Burkitt’s lymphoma cells, and hairy cells from patients resistant to IFNα therapy did not exhibit these features.

Effects upon the cytoskeleton are thought to be important actions of IFN in many cell types and have been shown to correlate with cellular differentiation and subsequent response to IFN (120). Ultrastructural studies of hematopoietic cells have revealed that normal and malignant plasma cells characteristically possess “bubbling” or “blebbled” surface membranes (121). Although IFNα-treated hairy cells do not appear to become morphologically identical to plasma cells, it is tempting to speculate that the appearance of areas of bubbling membrane on these cells represents progression towards plasmacytic differentiation. Previous studies demonstrating the synthesis of new organelles in cells from HCL patients responding to IFNα therapy lend support to this idea (122). The failure of these cells to become morphologically indistinguishable from plasma cells is consistent with the hypothesis that IFNα-induced hairy cell differentiation becomes arrested and that late-acting factors may be needed for terminal differentiation to occur. However, in most patients, partial differentiation may be sufficient to effectively control hairy cell proliferation.

Summary: Antiproliferative Effects of Interferon in Hairy Cell Leukemia

We have demonstrated that ultrastructural changes occur only in hairy cells displaying immunological modifications suggestive of cellular differentiation and that these changes correlate well with response to IFNα therapy. Affected cells exhibit altered expression of several antigens that have been implicated as components required for normal B-cell activation and proliferation (33, 123, 124). The effects of such changes upon the proliferative capabilities of hairy cells might be crucial for the favorable responses seen in HCL patients receiving IFNα to occur. In addition, the specificity of these immunological and morphological changes reflects both the unique sensitivity of hairy cells to IFNα and the inability of these cells to undergo the later stages of differentiation in a manner similar to that of other B-lymphocytes. The ability of IFNα to promote hairy cell maturation might explain the decreased growth factor responsiveness exhibited by IFNα-treated hairy cells. Whether these changes are the result of oncogenic regulation by IFNα has not yet been determined. However, the failure of IFNα to induce terminal differentiation in hairy cells might indicate the limits of IFNα as a single agent in HCL.

Concluding Remarks

Our extensive review of the literature, laboratory investigations, and large clinical experience with HCL patients allows us to draw several conclusions regarding the treatment of HCL and other malignancies with biological agents.

Mechanism of Interferon Action in Hairy Cell Leukemia. Hairy cell leukemia is a malignancy of partially activated B-lymphocytes displaying a mature phenotype characterized by positivity for HLA-DR, CD25, and tartrate-resistant acid phosphatase. B-lymphocytes at this stage of differentiation engage in clonal proliferation in response to multiple cytokine growth factors; correspondingly, hairy cell proliferation is uniquely dependent upon these same factors. B-cell growth is controlled in part by induction of differentiation, rendering cells less responsive to proliferative signals. Interferons have been demonstrated to produce such effects in normal and malignant lymphocytes (102, 104).

α-Interferon exerts direct effects upon the hairy cell, including modification of oncogene expression (107), induction of specific protein synthesis (125), and modulation of cytokine production (95). Present evidence indicates that these actions function to induce partial hairy cell differentiation, rendering the cell immune to growth factor stimulation, thereby inhibiting proliferation. The observed manifestations of this change include morphological alterations and a different immunophenotype, features which have been clinically correlated with favorable responses to IFNα therapy (62, 87, 116). Hence, IFNα acts as a cytokine in reducing the hairy cell index, not as a cytotoxic agent. Its actions upon the immune system have been shown to facilitate the effective elimination of infectious pathogens, but not of the hairy cell.

The unique sensitivity of the hairy cell to IFNα, which is not exhibited by cells from CLL and related lymphoproliferative disorders, can now be explained on the basis of their differing stages of maturation. B-cell growth factors are known to affect only cells in particular phases of development. The mechanisms underlying this differential sensitivity are not completely understood; however, they often depend upon activation/differentiation-dependent expression of growth factor receptors. CLL cells have been shown to possess type I interferon receptors at densities comparable to those of hairy cells (126), but their failure to express growth factor receptors susceptible to down-regulation by IFNα may indicate that these cells do not depend upon these factors for growth promotion. It is therefore not surprising that IFNα, despite its ability to alter cellular processes in leukemic cells from CLL, does not affect cellular growth to any great extent in CLL. This concept is supported by several studies demonstrating that hairy cells taken from patients resistant to IFNα therapy display atypical immunophenotypes, placing them in differentiation stages of greater or lesser maturity than that of the typical hairy cell. Interestingly, cells from neither CLL nor the two major recognized variant (resistant) HCL types express the IL-2R (127, 128). This finding probably indicates that the response to IFNα therapy depends upon interruption of IL-2-sustained hairy cell proliferation or that the presence of CD25 marks a hairy cell differentiation stage in which receptors for other essential growth factors are expressed and down-regulated by IFNα.

Implications for the Biotherapy of Hairy Cell Leukemia and Other Malignancies. Clinical trials using IFNα against a wide variety of malignancies have shown that favorable responses to therapy are only infrequently seen when IFNα is used in high...
MECHANISM OF INTERFERON IN HAIRY CELL LEUKEMIA

Doses as a chemotherapeutic agent rather than as a cytokine. The evidence presented in this review supports the idea that the beneficial effects of IFNα in cancer are more likely to be due to the role of IFNα as a cytokine rather than a cytotoxic agent. However, because IFNα acts as an antiproliferative biological response modifier in HCL, complete bone marrow remissions are quite uncommon. IFNα has not been shown to cause irreversible damage to hairy cell progenitors; as a result, nearly all HCL patients eventually relapse after termination of IFNα therapy (21). In addition, some patients initially responding to IFNα develop resistance to its effects; these cases may involve hairy cells that partially differentiate in response to IFNα but for some reason continue to proliferate (62). In vitro studies have clearly demonstrated that IFNα is not capable of inducing terminal differentiation in hairy cells. The high relapse rates seen in HCL and the development of acquired resistance in some HCL cases indicate that the inability of IFNα to irreversibly harm hairy cell progenitors and its failure to induce terminal differentiation may define the limits of IFNα therapy in HCL.

Although these limitations may apply when IFNα is used as a lone agent, the possibility of overcoming resistance to IFNα using logical combined biotherapy has not yet been assessed. The recent demonstration (129) that IFNα induces receptors for melanocyte-stimulating hormone on melanoma cells, causing them to differentiate in response to melanocyte-stimulating hormone, displays the rationale behind this approach and illustrates the true potential of cancer biotherapy. IFNα-resistant hairy cells have been demonstrated to possess greater or lesser degrees of maturity in comparison to typical hairy cells; we and others (127, 128) have suggested that this difference may form the basis of the resistant phenotype. It is therefore reasonable to speculate that the addition of early-acting cytokines targeted to the appropriate level of hairy cell maturity might cause less mature hairy cells to become sensitive to IFNα, while agents associated with late events in B-cell development might induce further differentiation in IFNα-treated resistant cells. Our growing understanding of the nuclear and cytosolic events that characterize B lymphocyte differentiation makes HCL an excellent model in which to examine logical combined cancer biotherapy, the “fourth arm” of modern cancer treatment (130).

In addition, the demonstrated effects of IFNα upon the lymphocytic cell cycle suggest that further elucidation of these pathways may eventually allow us to temporally coordinate chemotherapy with biotherapy. Following multidisciplinary studies of oncogene expression, growth factor responsiveness, and tumor cell differentiation, these approaches might be rationally and effectively utilized in other malignancies as well.

References

MECHANISM OF INTERFERON IN HAIRY CELL LEUKEMIA


Mechanism of Interferon Action in Hairy Cell Leukemia: A Model of Effective Cancer Biotherapy

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