Interferon gamma Increases In Vitro and In Vivo Expression of C1 Inhibitor

By Ghanushyam D. Heda, Stefania Mardente, Louis Weiner, and Alvin H. Schmaier

C1 inhibitor (C1 INH) is the major protease inhibitor of the first components of the classic complement system and of the proteases of the Hageman factor pathways. Since C1 INH may modulate inflammatory reactions associated with complement and contact system activation, we sought to determine if the cytokine gamma interferon (IFN-γ) could modulate C1 INH production. Initial studies investigated the effect of IFN-γ on the molecular and protein expression of C1 INH in human erythroblast leukemia (HEL) cells. HEL cells constitutively expressed the 2.1 kb mRNA for C1 INH. IFN-γ (50 to 1,000 U/mL), but not interferon alpha or beta, increased twofold the amount of C1 INH mRNA expressed within HEL cells. Similarly, this cytokine increased HEL cell C1 INH synthesis of a 105 Kd protein 10-fold, from 1.9 ± 0.5 μg C1 INH antigen per 10^9 cells (mean ± SEM) to 19 ± 8 μg/10^9 cells in 9 days. C1 INH produced by HEL cells after IFN-γ stimulation had fully intact kallikrein neutralizing activity. Moreover, conditioned media of IFN-γ-treated HEL cells accumulated more secreted C1 INH in 8 days (6.7 μg/mL/10^8 cells) than untreated cells (0.6 μg/mL/10^8 cells). Additional studies were done on plasma specimens from 22 patients with metastatic colorectal carcinoma who received IFN-γ daily for 4 days by intravenous infusion. Before treatment, the mean ± SEM C1 INH levels in these patients was 438 ± 16 μg/mL. At day 10 from the start of the infusion, the plasma C1 INH in these patients increased to 586 ± 32 μg/mL (P < .0001). The extent of rise of plasma C1 INH after IFN-γ treatment was independent of dose from 0.01 to 40 U/m2. After 30 days, the mean plasma C1 INH levels decreased to 502 ± 27 μg/mL. These combined studies indicate that IFN-γ can increase C1 INH protein expression in vitro and in vivo.

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MODULATION OF proteolytic reactions of the complement and contact phase systems within the vasculature may require the production and/or expression of inhibitors of these proteolytic enzymes at the sites of inflammation. In support of this hypothesis, monocytes, platelets, and endothelial cells have been found to contain C1 inhibitor (C1 INH). Monocytes, U937 cells, and endothelial cells have been shown to synthesize C1 INH. Interferon gamma (IFN-γ) has been shown to increase protein synthesis of C1 INH in endothelial cells, monocytes, and synovial fibroblast-like cells. Since platelets and monocytes contain C1 INH, we sought to determine if cells of the human erythroblast leukemia (HEL) cell line, which have both platelet and monocyte features, can also produce C1 INH. Further, we sought to determine if IFN-γ can modulate HEL cell expression of C1 INH and whether IFN-γ can modulate C1 INH in vivo. These studies indicate that IFN-γ can increase the constitutive molecular and protein expression of HEL cell C1 INH. Further, IFN-γ is able to elevate the plasma level of C1 INH in individuals without C1 INH deficiency.

METHODS

Materials. Goat antisera monospecific for human C1 INH was purchased from Atlantic Antibodies (Scarborough, ME). When this antisera was incubated with plasma, a single precipitin arc that showed complete identity with purified C1 INH was evident using double immunodiffusion, as previously reported. [35S]methionine (1,000 Ci/mmol) was obtained from ICN Pharmaceuticals (Irvine, CA), and [32P]dCTP (3,000 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Nylon membrane (0.2-μm pore size) was purchased from ICN. All reagents and molecular weight standards for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from BioRad Corp (Richmond, CA). The chromogenic substrate H-D-Pro-Phe-Arg-pNA (S2302) was purchased from Sigma Chemical Corp (St Louis, MO) and were the best grade available.

Plasma and proteins. Pooled normal plasma (lot 313D) was purchased from George King Biomedical, Inc, Overland Park, KS. C1 INH was purified from plasma by polyethylene glycol precipitation, DEAE-cellulose chromatography, concanavalin A-sepharose affinity chromatography, and gel filtration on sepharose CL-6B, as previously reported. C1 INH migrated predominantly with an apparent molecular mass of 105 Kd when analyzed by SDS-PAGE under nonreducing conditions. Prekallikrein was purified by the method of Scott et al. Recombinant IFN-γ with specific activity of 1.8 x 10^10 U/mg was generously supplied by Genentech, Inc, South San Francisco, CA. Recombinant interferon alpha (IFN-α; 2 x 10^10 U/mg) was purchased from Schering Corp, Kenilworth, NJ. Recombinant interferon beta (IFN-β; 5.4 x 10^10 U/mg) was provided by Trion Bioscience, Inc, Alameda, CA. Purified α2-macroglobulin was provided by Dr Marc Schapira, Vanderbilt University, Nashville, TN.

Functional and immunochemical assays. The activity of purified C1 INH was measured indirectly by obtaining the pseudo-first order rate constant for the neutralization of purified kallikrein using the chromogenic substrate H-D-Pro-Phe-Arg-pNA, as previously reported. Purified C1 INH antigen was also measured by radial immunodiffusion. The C1 INH content of washed, solubilized HEL cells was 3517±240 pg/mL.

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cells and their conditioned media were determined by a competitive enzyme-linked immunosorbent assay (CELSA), done as previously reported using a polyclonal antibody directed to C1 INH. In this assay, purified C1 INH antigen, measured as protein, produced competition inhibition curves that were superimposable onto curves produced by plasma containing equal amounts of C1 INH antigen and plasma from which all detectable C1 INH had been removed by immunoadsorption and then reconstituted with purified C1 INH. The antibodies used in these studies did not show any crossreactivity on immunodiffusion, immunoblot, or CELISA with any preparation of human and bovine serum albumin or fetal bovine serum used in these studies. Antiserum to human α1-antitrypsin was provided by Dr Frederick Kueppers, Temple University, Philadelphia, PA. Antigenic α1-antitrypsin was measured in plasma by radial immunodiffusion using pooled normal plasma as the standard of 1 U/mL.

Prekallikrein was measured by means of a spectrophotometric assay using the substrate H-D-Pro-Phe-Arg-pNa, as previously reported. The antibodies used in these studies did not show any crossreactivity on immunodiffusion, immunoblot, or CELISA with any preparation of human and bovine serum albumin or fetal bovine serum used in these studies. Only HEL cell lysates whose kallikrein neutralizing activity was greater than 85% neutralized by antibody to C1 INH were used to calculate C1 INH functional activity according to the following procedure. To inactivate any possible HEL cell α1-macroglobulin, 48 µL of HEL cell lysate was incubated with 12 µL 0.2 mol/L methyamine, pH 7.4, for 2 hours at 23°C. Preliminary studies showed that this amount of methyamine was sufficient to inhibit an additional 200 µg/mL pure α1-macroglobulin added to an HEL cell lysate. At the time of assay, 10 µL purified kallikrein (1.5 × 10−8 mol/L) were added to the mixture, and at precise intervals (1, 3, 5, 8, 11, 15, 20, 25, and 35 minutes), 5 µL of the mixture were removed and the residual kallikrein-dependent amiloride activity was determined as previously reported. The amount of C1 INH activity in the HEL cell lysate was calculated using the integrated second-order reaction equation: (1/INH – K) ln [(K(INH – K ⋅ INH)/(K – K ⋅ INH)) = k't, where t is the time of the incubation, k' is the second order rate constant for C1 INH inhibition of kallikrein (1.7 × 10−2 mol−1 s−1). K is the concentration of C1 INH. The next day, Pansorbin was added and the resultant precipitates were washed by centrifugation. The Pansorbin pellets were then incubated with 1.0 mol/L SDS, 2% β-mercaptoethanol in 0.01% Tris, pH 6 to 8, boiled for 5 minutes, and analyzed using 8% SDS-PAGE, according to the procedure of Laemmli. The gels were treated with Enhance (DuPont, Wilmington, DE) before drying. Autoradiography was done using Dupont Cronex II film with intensifying screens at −80°C.

**RNA isolation and Northern blot analysis.** Total cellular RNA was prepared from unstimulated HEL cells or HEL cells that had been incubated with IFN-γ (1,000 U/mL) for 16 hours using guanidine isothiocyanate and CsCl gradient centrifugation. RNA was quantified by optical density at 260 nm. For Northern blot analysis, total cellular RNA (20 µg) was electrophoresed in 1% agarose-formaldehyde gels and transferred onto nitrocellulose filters.

A CDNA probe to human C1 INH was provided by Dr Susan C. Bock, Temple University, Philadelphia, PA. A mixture of a 0.5 and 1.3 kb fragments derived from this plasmid cDNA by a double digest using EcoRI and HindIII was used as a probe for the quantity of total RNA added to the gel. A cDNA probe to plasminogen activator inhibitor I was provided by Dr T-C Wun, Monsanto Chemical Corp, St Louis, MO. All these probes were radiolabeled with [32P]dCTP by the random primer technique. Studies for protease nexin 1 mRNA were done by D. Guttridge, University of California at Irvine, Irvine, CA, using a 603 bp [32P]-radiolabeled riboprobe produced from a cDNA corresponding to amino acids 150 to 350. Hybridization was carried out overnight in 6X SSC, 0.1% SDS; twice with 1X SSC, 0.1% SDS; and once with 0.1X SSC, 0.1% SDS; all at 42°C. After hybridization, the filters with C1 INH and plasminogen activator inhibitor I probes were washed twice with 2X SSC, 0.1% SDS; twice with 1X SSC, 0.1% SDS; and once with 0.1X SSC, 0.1% SDS; all at 42°C for 30 minutes each. The filters with the actin probe were washed once with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS, both at 65°C. The filters were then exposed to Dupont Cronex II film with intensifying screens at −80°C.

**Patient studies.** Plasma samples were collected in 3.8 g % sodium citrate (1 part anticoagulant to 9 parts blood) from 22 patients with metastatic colorectal carcinoma who were enrolled in Phase 1 trials to assess the toxicity and efficacy of IFN-γ for treatment of their malignancy. Most of these patients were either asymptomatic or mildly symptomatic. All of these patients gave full written informed consent before entering the study. The patients were treated with variable doses of IFN-γ (0.001 to 60 × 10⁶ U/m²) intravenously by 4-hour infusions daily for 4 consecutive days. Plasma samples were collected before therapy and on day 10 and 30 after therapy was started. Only patients who contributed plasma for personal use only.
specimens on all 3 days were included in the analysis. The data were analyzed by grouped paired \( t \) test.

**RESULTS**

Initial investigations determined that HEL cells constitutively expressed the 2.1 kb mRNA for C1 INH (Fig 1). When HEL cells were stimulated with IFN-\( \gamma \) (1,000 U/mL) for 16 hours, the amount of mRNA for C1 INH increased. In contrast, equal amounts of actin-\( \gamma \) mRNA were expressed by unstimulated and IFN-\( \gamma \)-stimulated cells. IFN-\( \gamma \) specifically increased C1 INH mRNA expression. Neither IFN-\( \alpha \) nor -\( \beta \) were able to obviously elevate HEL cell C1 INH mRNA expression (data not shown). However, the extent of elevation of HEL cell C1 INH mRNA expression by IFN-\( \gamma \) was only about twofold greater than unstimulated cells after treatment of the HEL cells with 50 to 1,000 U/mL IFN-\( \gamma \) (data not shown).

Further investigations were done to determine if IFN-\( \gamma \) could modulate C1 INH protein expression in HEL cells. IFN-\( \gamma \) increased the concentration of C1 INH expressed within HEL cells (Fig 2A). Unstimulated HEL cells contained 1.9 \( \pm \) 0.5 \( \mu \)g C1 INH/10\(^6\) cells (mean \( \pm \) SEM). After continuous exposure to IFN-\( \gamma \) (100 U/mL), the HEL cell C1 INH content progressively increased \((P < .01)\) to 10-fold the untreated cells' concentration (19.1 \( \pm \) 8 \( \mu \)g C1 INH/10\(^6\) cells) at 8 days. In addition to increasing the HEL cell content of C1 INH, IFN-\( \gamma \)-stimulated HEL cells secrete more C1 INH (Fig 2B). After 4 days, IFN-\( \gamma \)-treated HEL cells had a significant increase (3.0 \( \pm \) 1 \( \mu \)g C1 INH/mL conditioned media from 10\(^6\) cells [mean \( \pm \) SEM]) in the concentration of C1 INH antigen in their conditioned media compared with that seen in the media of untreated cells (0.4 \( \pm \) 0.04 \( \mu \)g C1 INH/mL conditioned media from 10\(^6\) cells) (data not shown). After 8 days of treatment, IFN-\( \gamma \)-treated HEL cells accumulated more secreted C1 INH (6.7 \( \mu \)g/mL/10\(^8\) cells) than untreated cells (0.6 \( \mu \)g/mL/10\(^8\) cells).

The C1 INH immunoprecipitated from the conditioned media of metabolically labeled HEL cells was 105 Kd (Fig 3). When the same number of cells were treated with IFN-\( \gamma \), the amount of immunoprecipitable, metabolically labeled C1 INH increased. The IFN-\( \gamma \)-stimulated HEL cell induction of C1 INH produced a functionally active form of the protein. Freeze-thaw lysates of HEL cells stimulated with IFN-\( \gamma \) (100 U/mL) for 4 days had kallikrein neutralizing activity equivalent to 15.3 \( \pm \) 7 \( \mu \)g C1 INH/10\(^6\) cells (mean \( \pm \) SD, \( n = 6 \)) (Table 1). The kallikrein neutralizing activity of the HEL cell lysates was mostly C1 INH, because pretreatment of the lysates with a polyclonal antibody to C1 INH decreased 88% of their inhibitor activity to 1.9 \( \pm \) 1 \( \mu \)g C1 INH/10\(^6\) cells (\( n = 3 \)). Although most of the kallikrein neutralizing activity of IFN-\( \gamma \)-treated HEL cells was credited to C1 INH, unstimulated HEL cells were found to have similar levels of kallikrein neutralizing activity as the IFN-\( \gamma \)-treated HEL cell lysates (Table 2). Although 88% of IFN-\( \gamma \)-treated HEL cell kallikrein neutralizing activity was inhibited by antibody to C1 INH, virtually none of the kallikrein neutralizing activity in unstimulated HEL cells was inhibited by antibody to C1 INH (Table 2). Alternatively, 84% of the kallikrein neutralizing activity of unstimulated HEL cells lysates, similar to the kallikrein neutralizing activity of IFN-\( \gamma \)-treated cells (data not shown), was eliminated by treatment of the material with chloroform. The specific protease inhibitor that accounted for the kallikrein neutralizing activity in unstimulated HEL cells was not identified. The kallikrein neutralizing activity of lysates from unstimulated HEL cells was the same, regardless of whether or not the lysate was treated with methyamine. Moreover, neither plasminogen activator inhibitor 1 nor protease nexin I mRNA were found in unstimulated or IFN-\( \gamma \)-treated total RNA from HEL cells.

Additional studies were done to determine if IFN-\( \gamma \) could increase plasma C1 INH levels in vivo. Plasma samples from 22 patients with metastatic colorectal carcinoma who had received IFN-\( \gamma \) as part of Phase 1 trials were studied for C1 INH antigen (Fig 4A). Before the infusion, the mean \( \pm \) SEM C1 INH level in these patients was 438 \( \pm \) 16 \( \mu \)g/mL. At day 10 after the start of the administration of the IFN-\( \gamma \), the plasma C1 INH level in these patients increased to 586 \( \pm \) 32 \( \mu \)g/mL \((P < .0001)\). After 30 days from the start of the infusion, the mean plasma C1 INH levels in these patients decreased to 502 \( \pm \) 27 \( \mu \)g/mL \((P < .01)\) when compared with day 10; \( P > .1 \) when compared with day 0. Even though there was a wide range of doses of IFN-\( \gamma \) used to treat these patients, there was no relation between the size of the dose of IFN-\( \gamma \) and the extent of elevation of the patient's plasma C1
Fig 2. (A) HEL cell C1 INH content. HEL cells were washed with sterile 0.01 mol/L phosphate, 0.15 mol/L NaCl, pH 7.4 and were resuspended in fresh media containing 100 U/mL IFN-γ. The cells were maintained in the same media for the time indicated. On each of the days (0 to 8), aliquots of the cells were removed, washed by centrifugation, and solubilized with 0.5% Triton X-100. The C1 INH content in these lysates was determined by CELISA as indicated in Methods. The graphed results represent the mean ± SEM of three complete experiments with cells treated in this fashion. (B) HEL cell secretion of C1 INH. The conditioned media of HEL cells continuously stimulated with IFN-γ (100 U/mL) for 1 to 8 days were collected and assayed for C1 INH antigen by CELISA. The values plotted represent the mean ± SEM of three complete experiments with IFN-γ-treated HEL cell conditioned media.

INH level (Fig 4B). The rise in these patients’ C1 INH levels after IFN-γ treatment was not just due to the course of their illness. Their α1-antitrypsin levels, although the baseline was also elevated over normals, remained stable at 2.8 ± 0.3 U/mL, 2.65 ± 0.2 U/mL, and 2.83 ± 0.3 U/mL on days 0, 10, and 30, respectively, of the treatment period.

**DISCUSSION**

The finding that HEL cells constitutively express C1 INH message and protein was expected, since this protein is present in platelets, endothelial cells, and monocytes, and because this cell line expresses many features characteristic of megakaryocytes and monocytes. The ability of IFN-γ to increase the protein expression of C1 INH in HEL cells is consistent with previous studies with endothelial cells, monocytes, synovial fibroblasts, and skin fibroblasts (Figs 2 and 3). The rise in these patients’ C1 INH levels after IFN-γ treatment was not just due to the course of their illness. Their α1-antitrypsin levels, although the baseline was also elevated over normals, remained stable at 2.8 ± 0.3 U/mL, 2.65 ± 0.2 U/mL, and 2.83 ± 0.3 U/mL on days 0, 10, and 30, respectively, of the treatment period.

**Table 1. C1 INH Activity of IFN-γ-Treated HEL Cells**

<table>
<thead>
<tr>
<th>Sample</th>
<th>µg/C1 INH Activity/10⁶ Cells</th>
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<tbody>
<tr>
<td>HEL cell lysates (6)</td>
<td>15.3 ± 7</td>
</tr>
<tr>
<td>Antibody-treated HEL cell lysates (3)*</td>
<td>1.9 ± 1</td>
</tr>
</tbody>
</table>

*Antibody treatment consisted of making a supernatant of an HEL cell lysate 1 mg/mL with a polyclonal antibody to C1 INH and incubating the specimen overnight at 4°C. The antibody-treated HEL cell material was then assayed for kallikrein neutralizing activity, as indicated in Methods.

**Fig 3.** Immunoprecipitation of conditioned media from metabolically labeled HEL cells. Methionine-starved HEL cells had their media supplemented with [35S]-methionine and were grown in the absence or presence of IFN-γ (100 U/mL) for 48 hours. Immunoprecipitates were prepared as described in Methods. Conditioned media from unstimulated (A) and IFN-γ-treated (B) HEL cells were immunoprecipitated with antibody to C1 INH (AC1INH) or normal goat IgG (IgG). The figure is a photograph of an autoradiogram of an 8% SDS-PAGE of the immunoprecipitated radioactivity. The numbers to the right of the gel are molecular mass standards in kilodaltons. The mark (C1 INH) on the left of the gel is the migration of purified C1 INH on the same gel.
HEL CELLS EXPRESS C1 INHIBITOR

The present study expands the list of cell lines that produce C1 INH and that are responsive to IFN-γ. Within 16 hours of IFN-γ treatment, there was a distinct increase in the mRNA coding for C1 INH (Fig 1). This increase in C1 INH message in the HEL cells produced a significant increase in the concentration of synthesized C1 INH secreted into the conditioned media after only 4 days of continuous IFN-γ treatment (Figs 2 and 3).

The 10-fold increase after IFN-γ treatment in the concentration of C1 INH at 8 days in both the HEL cell lysate and its conditioned media over unstimulated control material suggest that this cell line could be used to produce large in vitro quantities of C1 INH for replacement therapy. This idea is enhanced by the finding that the C1 INH produced by the HEL cells is the same size (105 Kd) as normal plasma C1 INH (Fig 3). This finding suggests that the HEL cell C1 INH may have a similar glycosylation as normal plasma C1 INH. Further, the HEL cell C1 INH from IFN-γ-treated cells retained its functional ability to neutralize kallikrein over time: the higher the lysate, the more kallikrein neutralizing activity of unstimulated HEL cells indicates that it was not α2-macroglobulin.14 The lack of evidence for plasminogen activator inhibitor 1 or protease nexin I mRNA in 20 µg of unstimulated HEL cell total RNA suggests that these two other recognized kallikrein inhibitors are also not accounting for this activity.31,32 However, the finding that chloroform almost completely neutralized the kallikrein inhibiting activity of the HEL cell lysate suggests that the inhibitor may have structural similarity to C1 INH.33

increase the molecular expression of C1 INH mRNA in human skin fibroblasts, monocytes, and endothelial cells.4,29,30

Table 2. Kallikrein Neutralizing Activity of HEL Cells

<table>
<thead>
<tr>
<th>Sample (no. of experiments)</th>
<th>Slope of Kallikrein Neutralization (x 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated HEL cell lysates (8)</td>
<td>22 ± 5.8</td>
</tr>
<tr>
<td>Antibody-treated unstimulated HEL cell lysates (3)</td>
<td>22 ± 9.5</td>
</tr>
<tr>
<td>Chloroform-treated unstimulated HEL cell lysates (2)</td>
<td>3.5</td>
</tr>
<tr>
<td>IFN-γ-treated HEL cell lysates (3)</td>
<td>21 ± 4.1</td>
</tr>
<tr>
<td>Antibody-treated IFN-γ-treated HEL cell lysates (3)</td>
<td>2.5 ± 1.25</td>
</tr>
</tbody>
</table>

HEL cells were grown for 4 days in the absence or presence of 100 U/mL IFN-γ. After washing by centrifugation five times in 0.01 mol/L sodium phosphate, 0.15 mol/L NaCl, pH 7.4, containing 0.2% bovine serum albumin, the final pellet was resuspended at 10^6 cells/mL. The cell suspension was frozen at −70°C and thawed at 37°C five times, and the 10,000g supernatant was obtained.

The kallikrein neutralizing activity of the HEL cell lysate was determined by noting the decrease in the rate of hydrolysis of the chromogenic substrate at precise time intervals (0.5, 1.5, 3.5, 5, and 8 minutes) after the addition of purified kallikrein. The change in the rate of hydrolysis of the substrate is expressed as the slope of decreasing kallikrein activity over time: the higher the slope, the more kallikrein neutralizing activity of the lysate. Values represent the mean ± SD.

†Antibody treatment consisted of making a supernatant of an HEL cell lysate 1 mg/mL with a polyclonal antibody to C1 INH and incubating the specimen overnight at 4°C.

‡Chloroform treatment was done as previously reported16,33 by mixing an equal volume of ice cold chloroform with the HEL cell lysate, vortexing for 1 minute, and then centrifuging at 10,000g for 4 minutes. The supernatant of the material was used for further studies.

抑制剂(s) in unstimulated HEL cells lysates. This inhibitor(s) was downregulated by IFN-γ while C1 INH expression in the same cells was being upregulated. The fact that methylamine did not neutralize the kallikrein inhibitor in unstimulated HEL cells indicates that it was not α2-macroglobulin.14 The lack of evidence for plasminogen activator inhibitor 1 or protease nexin I mRNA in 20 µg of unstimulated HEL cell total RNA suggests that these two other recognized kallikrein inhibitors are also not accounting for this activity.31,32 However, the finding that chloroform almost completely neutralized the kallikrein inhibiting activity of the HEL cell lysate suggests that the inhibitor may have structural similarity to C1 INH.33

![Fig 4. (A) IFN-γ increases plasma C1 INH levels. Twenty-two cancer patients were treated with variable intravenous doses of IFN-γ daily for 4 consecutive days. Samples obtained at baseline (0) and at 10 and 30 days after the start of IFN-γ were stored at −70° before assay. The bar graph represents the mean ± SEM of samples collected from 22 patients at each of the indicated days. (B) Influence of IFN-γ dose on plasma C1 INH levels. The plasma C1 INH levels were measured in patients on day 0 and day 10. Each bar graph at every dose (0.01 to 40 U/m²) represents mean values from three different patients.](image-url)
Besides the potential in vitro use of INF-γ to induce C1 INH production in HEL cells, INF-γ may be useful in vivo to increase plasma C1 INH levels in patients with hereditary angioedema. Most patients with hereditary angioedema studied to date appear to have an expression defect for C1 INH rather than a deficient/defective gene. In vitro INF-γ treatment of monocytes in culture from patients with hereditary angioedema is effective in increasing the gene and protein expression of C1 INH in these cells. Our studies on serial plasma specimens from 22 cancer patients who had received INF-γ in Phase I trials indicate that this cytokine can significantly increase plasma levels of C1 INH in vivo (Fig 4A). The rise in these patients' C1 INH levels was not just due to their illness, because their α1-antitrypsin levels were not elevated after treatment. The degree of C1 INH elevation is striking for two reasons: INF-γ was able to elevate C1 INH levels 150 μg/mL above levels that were already high, and the C1 INH values were elevated for at least 6 days after the completion of the infusion of INF-γ. The high level of C1 INH in these patients before the infusion is characteristic of this protein being an acute phase reactant. The concomitant finding that α1-antitrypsin was also elevated supports this assessment. Although we are cautious in extrapolating the results of clinical data from cancer patients to individuals with hereditary angioedema, an increase in C1 INH levels of 150 μg/mL or 34% over pretreatment values after completion of an INF-γ infusion may ameliorate clinical symptoms associated with C1 INH deficiency.

Although INF-γ infusion may be associated with many side effects, there was no relation between the doses of INF-γ infused and the extent of rise in patient plasma C1 INH levels (Fig 4B). Therefore, very small doses of INF-γ, below the level that may induce side effects, may be sufficient to modulate an increase in the production of C1 INH in vivo. INF-γ exhibits a bell-shaped, dose-biologic response curve in its potentiation of human monocyte lysis of allogeneic tumor cells. Similar relationships have been seen with class II antigen expression, Fc receptor expression, and monocyte hydrogen peroxide production. Further studies are needed to determine if low dose INF-γ therapy can increase plasma C1 INH levels in some patients with hereditary angioedema and to determine if this increase in the level of C1 INH can ameliorate acute exacerbations of this disorder.

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REFERENCES

24. Ponte P, Gunning P, Blau H, Kedes L: Human actin genes are single copy for α-skeletal and α-cardiac actin but multicopy for β and γ-cytoskeletal genes: 3′ untranslated regions are isotype specific but are conserved in evolution. Mol Cell Biol 3:1783, 1983


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