The New England
Journal of Medicine

Volume 324 FEBRUARY 21, 1991 Number 8

A CONTROLLED TRIAL OF INTERFERON GAMMA TO PREVENT INFECTION IN CHRONIC GRANULOMATOUS DISEASE

THE INTERNATIONAL CHRONIC GRANULOMATOUS DISEASE COOPERATIVE STUDY GROUP*

Abstract *Background.* Chronic granulomatous disease is an uncommon inherited disorder of phagocytes in which defective production of the reactive intermediates of oxygen predisposes patients to recurrent and severe pyogenic infections. Evidence from in vitro and in vivo studies indicates that interferon gamma can partially correct the metabolic defect in phagocytes. We assessed the efficacy of interferon gamma in decreasing the frequency of serious infections in patients with this disease.

Methods. We conducted a randomized, double-blind, placebo-controlled study in 128 patients with chronic granulomatous disease (median age, 15 years). Patients received interferon gamma (50 μg per square meter of body-surface area) or placebo subcutaneously, three times a week for up to a year. The primary end point of the study was the time to the first serious infection, defined as an event requiring hospitalization and parenteral antibiotics. Measures of phagocyte function were also monitored.

Results. In terms of the time to the first serious infection, there was a clear benefit from interferon as compared with placebo ($P = 0.0006$). Of the 63 patients assigned to interferon, 14 had serious infections, as compared with 30 of the 65 patients assigned to placebo ($P = 0.002$). There was also a reduction in the total number of serious infections — 20 with interferon as compared with 56 with placebo ($P<0.0001$). Interferon was beneficial regardless of age, the use or nonuse of prophylactic antibiotics, and the mode of inheritance (X-linked or autosomal recessive). However, there were no significant changes in the measures of superoxide production by phagocytes. Interferon therapy was well tolerated, and there was no evidence of serious toxicity.

Conclusions. For patients with chronic granulomatous disease, interferon gamma therapy is an effective and well-tolerated treatment that reduces the frequency of serious infections. (N Engl J Med 1991; 324:509-16.)

CHRONIC granulomatous disease is a heterogeneous group of uncommon inherited disorders characterized by recurrent pyogenic infections that usually begin early in life and may lead to death in childhood.[1-3] Two thirds of the cases involve disease inherited according to an X-linked pattern, and one third follow an autosomal recessive pattern.[4,5] Although phagocytes from patients with chronic granulomatous disease ingest microorganisms normally, killing is deficient because the capacity of the phagocytes to produce superoxide and related microbicidal oxygen intermediates is impaired by NADPH oxidase deficiency.[6-9] This oxidase is a multicomponent complex that includes a unique membrane-bound cytochrome *b* and several cytosolic components. The cytochrome *b* is a heterodimer consisting of a 91-kd glycosylated heavy chain and a 22-kd light chain, termed "gp91-*phox*" (*ph*agocyte *ox*idase) and "p22-*phox*," respectively.[10,11] The gene for the heavy chain is the site of mutations responsible for X-linked chronic granulomatous disease.[12,13] A deficiency of a 47-kd cytosolic protein (p47-*phox*) required for oxidase activity[5,14] accounts for most of the autosomal recessive forms[14-18] of this disorder. Two rare subgroups of autosomal recessive chronic granulomatous disease are due to defects in the gene for the light chain of the cytochrome b[19] or a defect in the product of a gene encoding for a 67-kd cytosolic protein (p67-*phox*).[5,15,16,20,21]

The level of activity of NADPH oxidase, which is dormant in resting cells, is affected by exposure to priming substances, including cytokines[22-25] and bacterial lipopolysaccharides.[24] These agents do not directly activate the oxidase but serve to enhance its level of activity after stimulation. Experiments in both humans and mice have shown that interferon gamma is a principal macrophage-activating factor. Administering interferon gamma either in vivo or in vitro enhances both the production of reactive oxygen intermediates and the killing of bacterial and protozoal pathogens.[26] The use of interferon gamma in clinical trials in patients with lepromatous leprosy and cancer enhanced the capacity of circulating monocytes to secrete hydrogen peroxide.[25,27] Moreover, interferon gamma has recently been shown

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Supported in part by the National Institutes of Health and Genentech, Inc.

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to be a potentially important adjunct to conventional therapy for systemic leishmaniasis.[28]

When interferon gamma was added in vitro to granulocytes and monocytes from patients with various genetic types of chronic granulomatous disease, the defect in superoxide production was partially corrected in some patients' phagocytes.[29,30] Partial correction of the functional defect was also observed after treatment of patients with subcutaneous interferon gamma.[30,31] Since the treatment was well tolerated, a randomized, double-blind, placebo-controlled trial was designed to determine whether interferon gamma could decrease the frequency and severity of serious episodes of infection and improve chronic infectious and inflammatory conditions in patients with all genetic types of chronic granulomatous disease.

Methods

Patients

This trial involved 13 study centers in four countries. Patients who were enrolled had a confirmed diagnosis of chronic granulomatous disease before entry; the diagnosis was based on both abnormal results on neutrophil nitroblue tetrazolium slide testing (after stimulation with phorbol myristate acetate) and neutrophil superoxide production no more than 20 percent of normal. Other criteria for eligibility included preserved renal, hepatic, and hematologic function, a minimal life expectancy of three months, and the signing (by the patients or their parents or guardians) of a form giving informed consent. Patients who had previously received interferon gamma were eligible only if it had not been administered for at least three months, if the nitroblue tetrazolium or superoxide assay indicated a return to pretreatment status, and if no antibody to interferon gamma was detected in the serum. Patients were excluded if they had active infection requiring hospitalization and parenteral antibiotic therapy, severe cardiac disease, or confirmed seizure disorders, had not fully recovered from surgery, were pregnant or lactating, or were not practicing effective contraception while of childbearing age.

Patients were allowed to receive prophylactic antibiotic therapy — i.e., trimethoprim–sulfamethoxazole or dicloxacillin — according to a predetermined regimen that varied according to their age and history of drug allergy. Patients receiving corticosteroids were eligible only if they were both receiving 10 mg or less of prednisone daily and had been receiving the same dose for at least one month before entering the study. The use of other forms of immunotherapy, experimental drugs (including itraconazole, then experimental), and antiinflammatory compounds was not permitted during the study period.

Study Medication

Recombinant human interferon gamma (Genentech, South San Francisco; specific activity, approximately 3×10^7 U per milligram of protein) was supplied as a sterile solution ready for injection. Each vial contained either 0.2 mg of interferon gamma at a concentration of 0.2 mg per milliliter or placebo (interferon gamma diluent) and was labeled according to a centrally prepared randomization list.

Study Design

Patients were randomly assigned to treatment according to a dynamic, adaptive randomization scheme[32,33] stratified on the basis of the pattern of inheritance of the disease (X-linked or autosomal recessive), the use or nonuse of prophylactic antibiotics, the study-center institution (United States — a center of the National Institutes of Health or other centers; Europe — Amsterdam center or other centers), and the use or nonuse of corticosteroids. All patients, investigators, and trial coordinators were blinded to treatment assignment. The patients received interferon gamma or placebo by subcutaneous injection three times weekly (e.g., on Monday, Wednesday, and Friday) for up to 12 months unless unacceptable toxicity was observed. If the body-surface area of a patient was at least 0.5 m^2, the dose was administered on the basis of body-surface area, as 50 μg per square meter; if the body-surface area was less than 0.5 m^2, the dose was administered on the basis of body weight, as 1.5 μg per kilogram. Patients were carefully monitored on a periodic basis for clinical or laboratory evidence of toxicity related to interferon gamma. Toxicity was graded according to criteria specified in the protocol.

Monitoring for Efficacy

Serious Infections

The primary end point of this investigation was the time to serious infection, defined as a clinical event requiring both hospitalization and the administration of parenteral antibiotics. The time to the first serious infection was calculated as the number of days elapsed between the date of random assignment to treatment and the date of diagnosis of the infection. Patients were also followed up for the number and relative rate of serious infections. The assigned treatment was continued during periods of serious or minor infection. Patients discontinuing treatment at any time during the study period continued to be monitored to document the occurrence of the first serious infection (primary end point) and subsequent recurrent serious infections.

Chronic Clinical Conditions Related to Chronic Granulomatous Disease

Quantitative estimates of the severity of all clinical conditions related to chronic granulomatous disease were obtained within seven days before the initiation of treatment and at three-month intervals during the study. Two scoring systems were used: a visual-analogue system for scoring clinical severity, in which an absolute rating of the severity of each condition was recorded as a tick mark on a linear scale ranging from "absent" to "most severe," and a system for scoring clinical change ("change from first identification"), in which a seven-point scale ranging from −3 (severe worsening of >50 percent) to +3 (complete resolution) was used to assess change in each condition relative to its first occurrence. All evaluations of each patient were performed by the same physician.

Phagocyte-Function Assays

Neutrophil Nitroblue Tetrazolium Test

The neutrophil nitroblue tetrazolium test[34,35] was performed on fresh whole blood or isolated neutrophils. Equal volumes of cells were incubated in a solution of neutrophil nitroblue tetrazolium with 1 μg of phorbol myristate acetate per milliliter (stimulated incubation) and without it (unstimulated incubation). After incubation, the cells were fixed and counterstained with 1 percent safranin. The percentage of positive cells was determined microscopically. When the percentage of normal neutrophils is more than 95 percent, the neutrophil nitroblue tetrazolium is reduced.

Phagocyte Superoxide-Production Assay

The effect of interferon gamma on phagocyte respiratory-burst activity was determined by measuring the rate at which superoxide was generated by neutrophils in suspension (and, when available, monocytes) before and after stimulation with phorbol myristate acetate (660 ng per milliliter); this assay was performed before treatment and on days 4, 90, 180, 270, and 360. Superoxide was measured with a discontinuous, end-point assay (after 10 and 60 minutes) in which ferricytochrome *c* was used as the indicator of the presence or absence of superoxide dismutase.[36] Determinations were averaged before analysis. The two treatment groups were compared at each study visit with respect to differences in the rate of superoxide production (expressed as the number of nanomoles produced per 10 minutes).

Phagocytic Killing of Staphylococci

The effect of interferon gamma on bacterial killing of *Staphylococcus aureus* strain 502A[34] was measured for each assay time point (one

hour and two hours) and cell type (neutrophils and, when available, monocytes) before treatment and on days 4, 90, 180, 270, and 360. The percentage of bacterial killing in patients and normal controls was based on colony counts and bacteria levels in cell-free growth-control preparations. The two treatment groups were compared at each study visit for bacterial killing at each time point and for each cell type.

Statistical Analysis

The time to serious infection was the primary end point of this investigation. The trial was planned to have a minimal enrollment of 100 eligible patients. A patient receiving placebo and prophylactic antibiotics was expected to have at least one serious infection every two years. This ensured that a two-sided log-rank test with a level of significance of 0.05 would have a power of 0.85 to detect a fourfold reduction in the rate of serious infections (from one infection per two years to one per eight years) and a power of more than 0.75 to detect a threefold reduction.

The statistical methods employed included the Cochran–Mantel–Haenszel test, the Pearson chi-square test, analysis of variance, and the t-test.[37,38] The distribution of times to serious infection was compared between the groups by means of the log-rank test and the Cox proportional-hazards regression model.[39] The Cox proportional-hazards model was also used to determine the relative risk (hazard ratio) of serious infection in the treatment groups and the 95 percent confidence interval for the hazard ratio, and to perform all multivariate analyses. The Andersen–Gill generalization of the Cox regression model for the time to the first serious infection was used in the analysis of data on recurrent serious infection.[40,41] Curves showing the cumulative proportion of patients free of serious infection in each group were generated according to the Kaplan–Meier method.[42] Statistical analyses were computed with the procedures of SAS and BMDP (P1L and P2L).[43,44] All P values reported for this trial are two-sided.

The decision to terminate the trial early or to continue it was made during a planned interim analysis performed by an independent monitoring committee that had sole access to blinded data on efficacy. The committee was guided by the two-stage group sequential boundary of O'Brien and Fleming.[45] This analysis, based on an average duration of study treatment of 6.4 months and 68 cumulative patient-years, resulted in early termination of the study after interferon gamma therapy was demonstrated to have significant benefit with respect to the time to serious infection, as compared with placebo (20 patients in the placebo group vs. 7 in the interferon group, $P = 0.0036$).

Results

Characteristics of the Study Population

During a seven-month period a total of 135 patients were randomly assigned to receive either interferon gamma or placebo. Seven patients did not meet the eligibility criteria of the protocol, leaving 128 eligible patients for inclusion in the final analysis. Of the seven ineligible patients, two were using prohibited concomitant medication, two had serious active infections and no signed form giving informed consent, one had a history of seizure disorder and no signed form giving consent, and two declined to participate. As shown in Table 1, the two treatment groups were comparable at admission in age, sex, pattern of inheritance of chronic granulomatous disease, and use of prophylactic antibiotics and corticosteroids.

Table 1. Characteristics of Patients with Chronic Granulomatous Disease, According to Treatment Group.*

VARIABLE	INTERFERON	PLACEBO
No. of patients	63	65
Mean age ±SD (yr)	14.3±10.1	15.0±9.6
	no. of patients (percent)	
Sex		
Male	51 (81)	53 (82)
Female	12 (19)	12 (18)
Pattern of inheritance		
X-linked	45 (71)	41 (63)
Autosomal recessive	18 (29)	24 (37)
Prophylactic antibiotics		
Yes	56 (89)	55 (85)
No	7 (11)	10 (15)
Corticosteroids		
Yes	1 (2)	2 (3)
No	62 (98)	63 (97)
Monitoring institutions		
United States		
National Institutes of Health	15 (24)	11 (17)
Nine other centers	31 (49)	32 (49)
Europe		
Amsterdam	9 (14)	10 (15)
Two other centers	8 (13)	12 (19)
Patients completing treatment†	57	54

*There were no statistically significant differences between the groups in the variables shown.

†Six patients were removed from the interferon group early in the study because of fever (two), malaise or fatigue, rash, or colitis or by their request. Eleven patients were removed early from the placebo group by their request (five) or because of behavioral problems, allergic reactions, fungal skin infection, abdominal discomfort with diarrhea, or accidental unblinding (two).

Results of the Final Analysis

The final analysis was conducted with data collected on each patient through the end of the study, from the time of randomization until the date of the final blinded visit, including an additional 2.5 months of follow-up per patient beyond the date of the interim analysis. The average duration of treatment was 8.9 months, representing 95 patient-years. The median age of the patients was 15 years, and 37 patients were less than 9 years of age. One hundred eleven of the 128 eligible patients received the study treatment throughout the entire study period. There were no deaths.

Serious Infections

Thirty (46 percent) of 65 patients in the placebo group and 14 (22 percent) of the 63 in the interferon gamma group had at least one serious infection ($P = 0.0006$). When the time to the first serious infection in each group was plotted as a Kaplan–Meier curve (Fig. 1), a mean (±SE) of 77±0.06 percent of patients in the interferon group were free of serious infection 12 months after randomization, as compared with only 30±0.11 percent in the placebo group ($P = 0.0006$, by two-sided log-rank test). When these values were compared with those after six months (89±0.04 percent vs. 72±0.06 percent, respectively), the beneficial effect of interferon was clearly maintained with its continued administration during the first year. When the Cox regression model was used, with treatment as a covariate, the relative risk of a serious infection with interferon was only one third the risk with placebo, resulting in a 67 percent reduction in the relative risk of serious infection during interferon treatment (95 percent confidence interval for the

relative risk, 0.17 to 0.64; estimated log relative risk [±SE], −1.094±0.34).

Recurrent Serious Infections

Of the 30 patients assigned to placebo who had at least one serious event (infection), 5 had two events, 4 others had three, 1 had four, 1 had five, and 1 had seven. Of the 14 patients assigned to interferon gamma who had at least one serious event, 4 had a second event and 1 had three. Comparison of the total numbers and rates of serious infections observed to the end of study showed a significant difference between the groups (56 infections in the placebo group vs. 20 in the interferon group; $P<0.0001$ by partial-likelihood ratio test derived by fitting an Andersen–Gill regression model). This additional information on recurrent events reduced the variability in the estimate of treatment effect (relative risk, 0.33), reducing the 95 percent confidence interval (0.20 to 0.56) and thereby strengthening the evidence of a beneficial effect of interferon gamma therapy.

Analysis of the nature of serious events revealed that interferon gamma appeared to be most effective in reducing adenitis, abscesses, cellulitis, and pulmonary infections (Table 2). It was particularly noteworthy that only one patient receiving interferon had an episode of aspergillus pneumonia, as compared with four patients receiving placebo. In addition, patients receiving placebo required three times as many days of hospitalization for the treatment of clinical events as did patients receiving interferon (1493 vs. 497 days, respectively); the average stay was 32 days in the interferon group and 48 days in the placebo group ($P = 0.02$). Moreover, 13 patients in the placebo group were hospitalized for more than 28 days, as compared with 6 in the interferon group.

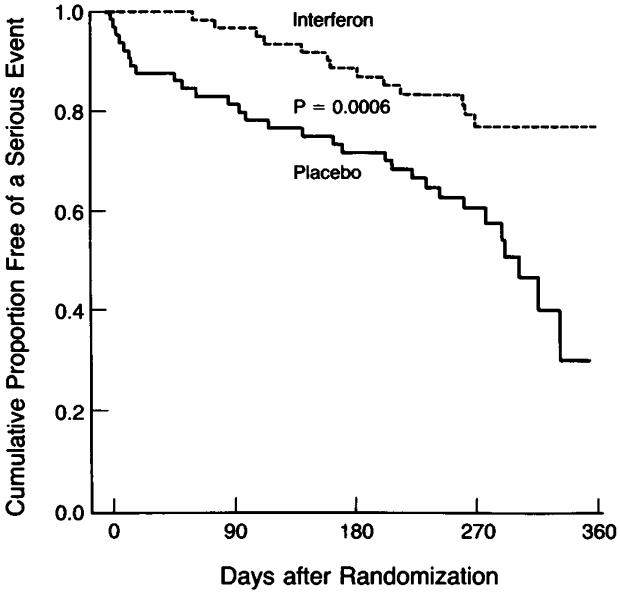

Figure 1. Cumulative Proportion of Patients in Each Treatment Group Who Were Free of Serious Events (Infections) during the Study.

The Kaplan–Meier curves show the time to the first serious event (infection) after randomization. In a Cox regression model in which treatment served as a covariate, there was a 67 percent reduction in the relative risk of serious infection in the interferon group (P value = 0.0006, by two-sided log-rank test).

Table 2. Clinical Events Requiring Hospitalization and Intravenous Antibiotics.

Event	Interferon (N = 14)	Placebo (N = 30)
	no. of events (no. of patients)	
Pulmonary infection*	7 (5)	12 (11)
Adenitis	2 (2)	11 (10)
Genitourinary tract infection or obstruction	1	5 (3)†
Hepatic infection	4 (3)‡	3 (3)
Sinusitis	0	2 (2)
Osteomyelitis or joint infection	1	2 (2)
Bacteremia	0	2 (2)§
Gastrointestinal obstruction or colitis	2 (2)	1
Abscess or cellulitis (not listed in other categories)	2 (2)	16 (14)
Other infections¶	1	1

*Includes two episodes of aspergillus pneumonia in one patient in the interferon group and four episodes of aspergillus pneumonia in four patients in the placebo group.

†One patient in the placebo group had an episode of epididymitis, an episode of ureteral obstruction, and an episode of urinary tract infection.

‡One patient in the interferon group was temporarily withdrawn from this treatment for five months, before having two episodes of hepatic abscess.

§In the placebo group only there was one episode of *Staph. epidermidis* bacteremia and one episode of enterococcal bacteremia.

¶In the interferon group there was one episode of viral syndrome due to catheter infection, and in the placebo group one episode of meningitis. There were three fungal infections in two patients in the interferon group and six fungal infections in six patients in the placebo group.

Subset Analyses

Kaplan–Meier curves were constructed for each category of the following five prognostic or potential confounding factors: pattern of inheritance (X-linked or autosomal recessive), use of prophylactic antibiotics (yes or no), institution (United States, National Institutes of Health; United States, other study center; Europe, Amsterdam; or Europe, other study center), sex, and age (younger, <10 years; or older, ≥10 years). The proportions of patients free of serious infection among those with X-linked disease and among those with autosomal recessive disease were similar, as were the proportions among male patients and female patients; however, patients less than 10 years of age and patients not taking prophylactic antibiotics had a slightly greater frequency of serious infections. The group of patients treated at European centers other than Amsterdam appeared to have a slightly greater proportion free of serious infection.

Analyses of the primary end point — the time to serious infection — were conducted to determine whether any of the prognostic factors or potential confounding variables had a significant interaction with treatment. Table 3 shows the estimated percent-

Table 3. Patients Free of Serious Infection 12 Months after Randomization, after Adjustment for Stratification Factors.

VARIABLE	NO. OF PATIENTS	PERCENT WITHOUT SERIOUS EVENT*	
		INTERFERON	PLACEBO
Patients at risk of first serious event (infection)	128	77	30
Adjusted for covariate			
Age			
<10 yr	52	81	20
≥10 yr	76	73	34
Pattern of inheritance			
X-linked	86	79	33
Autosomal recessive	42	71	39
Prophylactic antibiotics			
Yes	11	78	33
No	17	69	28
Sex			
Male	104	76	28
Female	24	83	56
Monitoring institution			
United States			
National Institutes of Health	26	73	19
Nine other centers	63	75	33
Europe			
Amsterdam	19	78	53
Two other centers	20	88	75

*Values are Kaplan–Meier estimates of the cumulative proportion of patients free of serious events (infection). Values for American institutions reflect status at 12 months, and values for European institutions reflect status at 10 months.

age of patients free of serious infection in each treatment group after 12 months, in relation to each of the five factors. Figure 2 shows the Kaplan–Meier curves for analyses according to the pattern of disease inheritance, use of prophylactic antibiotics, and age. In all cases interferon gamma had a benefit as compared with placebo. For example, 79 percent of the patients with X-linked disease and 71 percent of those with autosomal recessive disease in the interferon group were free of serious infection after 12 months, as compared with 33 percent of those with X-linked disease and 39 percent of those with autosomal recessive disease in the placebo group. It is notable that 78 percent of the patients receiving interferon gamma in conjunction with prophylactic antibiotics were free of serious infection after 12 months, as compared with 33 percent of the patients receiving placebo and antibiotics. The greatest effect of treatment with interferon gamma was observed among patients less than 10 years of age; 81 percent of those receiving interferon were free of serious infection after 12 months, as compared with 20 percent of those receiving placebo. The estimate of the percentage reduction in the relative risk of serious infection with interferon as compared with placebo was consistently around 67 percent.

Among the patients with X-linked disease, serious adverse events occurred in 19 receiving placebo and 9 receiving interferon gamma; among those with autosomal recessive disease, such events occurred in 11 receiving placebo and 5 receiving interferon. The relative risk of serious infection was 3.4 times greater among patients with X-linked disease who received placebo than among those who received interferon, and 2.3 times greater among patients with autosomal recessive disease. Even though the European centers had a lower overall rate of serious adverse events — probably because they had a shorter follow-up period and older cohorts — there was evidence of benefit from treatment with interferon gamma in both European and American institutions. The relative risk of serious infection was 3.5 times greater with placebo than with interferon in U.S. patients and 2.1 times greater in European patients.

The most striking finding regarding prognostic factors was the beneficial effect of interferon gamma in younger patients. Among patients less than 10 years old there were 15 serious infections with placebo and 6 with interferon, whereas among older patients there were 15 serious infections with placebo and 8 with interferon. Analysis of the time to a first serious infec-

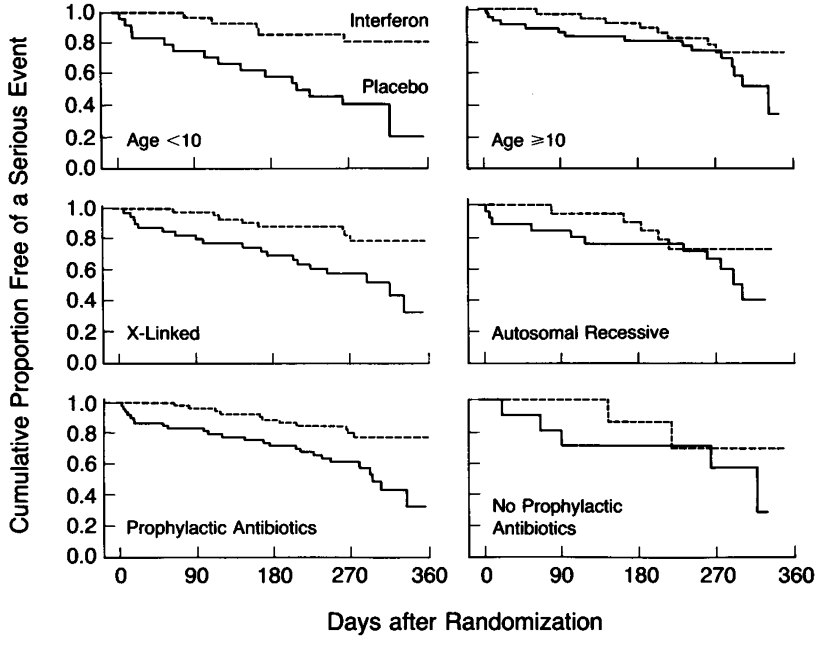

Figure 2. Proportion of Patients in Each Treatment Group Who Were Free of Serious Events, According to Age, Pattern of Disease Inheritance, and Use of Prophylactic Antibiotics.

For the subgroup <10 years old, the P value is 0.0005 and the relative risk (RR) 4.84; for the subgroup ≥10 years old, P = 0.135 and RR = 2.14. For the subgroup with X-linked disease, P = 0.0015 and RR = 3.38; for the subgroup with autosomal recessive disease, P = 0.206 and RR = 2.32. For the subgroup taking prophylactic antibiotics, P = 0.0015 and RR = 3.07; for the subgroup not taking antibiotics, P = 0.307 and RR = 2.37.

tion revealed that the relative risk of serious infection was 4.8 times greater with placebo than with interferon among the younger patients and 2.1 times greater among the older patients.

Chronic Conditions Related to Chronic Granulomatous Disease

The effect of interferon gamma on the relief and prevention of chronic conditions related to chronic granulomatous disease was assessed at each patient visit. These conditions included granulomatous obstruction of the gastrointestinal and genitourinary tracts, fistulas, abscesses, lymphadenitis, and dermatitis. This assessment failed to reveal any statistically significant benefit or deleterious effects of interferon on these conditions, as compared with placebo.

Phagocyte Function

No statistically significant differences were observed between the interferon and placebo groups in either superoxide production or bacterial killing at any of the study time points at any of the centers, as indicated by neutrophil assays performed in all patients and monocyte assays performed in less than 25 percent of the patients. Analysis of the pooled results from all centers gave results similar to those for individual centers. Cytochrome *b* levels were not routinely measured at all centers because of the technical difficulty and sensitivity of the assay. Where they were measured, however, no effect of interferon gamma on cytochrome *b* levels was observed (measured in <10 percent of all enrolled patients).

Adverse and Intercurrent Events

No serious or life-threatening toxic effects could be directly attributed to the administration of interferon gamma. The most common clinical reactions observed were fever, chills, headache, and erythema at the injection site (Table 4). The majority of reactions were mild, and concomitant administration of acetaminophen appeared to ameliorate most reactions. When interferon gamma was administered at bedtime, there were fewer reactions. Comparison of the incidence of adverse events according to age revealed a greater incidence of reactions in older patients. Constitutional symptoms occurred twice as frequently in patients 10 years old or older as in those less than 10 years old. In children 5 years old or younger, the incidence of adverse reactions was lower than in all the other patients. No adverse effects were observed on growth as determined by weight and height; no obvious effects on development were observed. Four patients were withdrawn from therapy because of toxicity related to interferon: one patient for skin rash, two for constitutional symptoms, and one for worsening granulomatous colitis. No effect of interferon on hematologic or biochemical indexes was observed. Elevation of hepatic enzyme levels was noted in 23 percent of patients receiving placebo and 16 percent of those receiving interferon (all elevations were less than three times base-line values). In addition, interferon gamma had no effects on the levels of rheumatoid factor or antinuclear antibodies, the sedimentation rate, thyroid function, or sex hormone levels. In 54 patients treated with interferon for whom paired blood specimens were available, antibodies against interferon gamma were not detected.

Table 4. Adverse Reactions during Study.

Reaction	Percent with Reaction		P Value*
	Interferon (N = 63)	Placebo (N = 65)	
Fever	52	28	0.01
Headache	33	9	0.001
Rash	17	6	NS
Chills	14	0	0.001
Injection-site erythema or tenderness	14	2	0.01
Fatigue	14	11	NS
Diarrhea	14	12	NS
Vomiting	13	5	NS
Nausea	10	2	NS
Weight loss	6	6	NS
Myalgia	6	0	NS
Inflammatory process requiring steroid therapy†	6	6	NS
Anorexia	3	5	NS
Arthralgia	2	0	NS
Injection-site pain	0	2	NS

*By two-sided Fisher's exact test for treatment-group differences. NS denotes not significant (P>0.05).

†In the interferon group, the clinical conditions included granulomatous colitis, gastric-outlet obstruction, ureteral obstruction with hepatic granuloma, and hepatic abscess with hepatic granuloma. In the placebo group, the clinical conditions included granulomatous colitis with antral obstruction, gastrointestinal and renal obstruction, ureteral obstruction, and iridocyclitis.

Discussion

In this double-blind, placebo-controlled study, subcutaneous injections of recombinant interferon gamma administered three times weekly reduced the frequency of serious infections in patients with chronic granulomatous disease. Interferon was beneficial regardless of the pattern of inheritance of disease, age, or use or nonuse of prophylactic antibiotics. Patients receiving interferon had a reduction in the frequency and total length of hospitalizations. Children less than 10 years of age benefited most from treatment and had fewer side effects. Treatment was not accompanied by toxic effects other than fever, mild headache, and myalgia, which were treated with acetaminophen. No antibodies to interferon gamma were detected.

Since chronic granulomatous disease is often diagnosed during the first few years of life, it is noteworthy that the treatment was well tolerated in patients of this age and was not associated with disturbances in growth and development as determined after 12 months of follow-up. It is especially encouraging that the ability of interferon gamma to reduce infection was most dramatic in children less than 10 years old.

Interferon gamma therapy was effective in all genetic types of chronic granulomatous disease. The precise molecular mechanisms by which it improves host de-

fense in this disorder remain unknown. In the present study, there appeared to be no correlation between the clinical benefit of interferon and the improvement in phagocyte function, in contrast to the results of previous studies.[29-31] In those studies, neutrophils and monocytes from patients with X-linked disease showed augmented superoxide production and cytochrome *b* gene expression after interferon gamma treatment in vitro or in vivo. Several patients with autosomal recessive chronic granulomatous disease who lacked either the p47 or the p67 cytosol oxidase component also had a slight improvement in superoxide production. The most likely explanation for this apparent discrepancy is that although the majority of patients with chronic granulomatous disease do not have improvement in respiratory-burst function, there are rare patients who have mutations that permit a phagocyte response to interferon gamma (e.g., gene-regulatory mutations). The clinical response of such patients may be due in part to the effect on the respiratory burst. In the majority of patients, however, interferon gamma may confer benefit by augmenting oxygen-independent antimicrobial pathways of phagocytes, which were not determined in this study. Another explanation is that the interferon improved the function of other components of the immune system (e.g., T or B cells). Yet another is that any interferon gamma–induced changes in superoxide production were below the level of detection of the assay for extracellular superoxide or that hydrogen peroxide (rather than superoxide) was preferentially generated by the slight enhancement of oxidase. Further study will be required to determine the effects of interferon gamma on oxygen-dependent and oxygen-independent microbicidal pathways. Molecular characterization of specific defects in a large number of patients within each genetic subgroup, and correlation of the defect with functional response to treatment, should also help define the mechanisms by which interferon gamma augments the host response.

Interferon gamma treatment was accompanied by minimal side effects. Chills, myalgias, and erythema were observed nearly exclusively in the interferon group. Fever, however, occurred in a third of the patients receiving placebo, as compared with half of those receiving interferon. Constitutional side effects were reduced by bedtime administration of interferon gamma with acetaminophen. Although this study establishes an effective dose, dose interval, and route of administration, further studies are planned to evaluate the effectiveness of giving subcutaneous injections weekly as compared with three times weekly.

This study shows that interferon gamma is an effective and safe therapy for patients with chronic granulomatous disease, since the drug substantially reduced the frequency of serious infections. The results strongly support the recommendation that interferon gamma be given to these patients as prophylaxis against infections, either alone or (preferably) in conjunction with prophylactic antibiotics. The potentially effective use of interferon gamma for other infectious diseases, such as leprosy[27] and leishmaniasis,[28] together with the realization that mechanisms other than the augmentation of superoxide production probably contribute to the mechanism of action of this agent, suggests that this cytokine may have general applications as an adjunct to conventional antimicrobial agents in the treatment of many types of infectious diseases.

We are indebted to B. Babior, M.D., Ph.D. (Scripps Clinic and Research Foundation, La Jolla, Calif.), R.B. Johnston, M.D. (Children's Hospital of Philadelphia), and S. Klebanoff, M.D. (University of Washington, Seattle), for their participation on the Independent Data Safety and Monitoring Committee; to D.W. Alling, M.D., and S.M. Banks, Ph.D. (National Institutes of Health, Bethesda, Md.), and S.A. Sherwin, M.D. (Genentech, S. San Francisco), for advice on study design and statistical analyses; and to E. De Carlo (National Institutes of Health), J. van Pelt and R. van Zweten (Academic Medical Center and Central Laboratory of the Netherlands Red Cross, Amsterdam), the staff of the Scripps Clinic General Clinical Research Center and S. Burke (La Jolla), I. Tanner and C. Erny (Kinderspital, Zurich), C. Brown and M. Burton (C.S. Mott Children's Hospital, University of Michigan, Ann Arbor), L.C. Hiebert and M. Dombrowski (Baylor College of Medicine, Texas Children's Hospital, Houston), L. Beyer (Children's Hospital of Los Angeles), A. Hansen (Hvidovre Hospital, Copenhagen), B.R. Michael (University of Washington), S. Martin and M. Reiter (Mount Sinai Medical Center, New York), G.J. Leitz, M.D. (Boehringer Ingelheim, Ingelheim, Germany), and P. Tufte, S. Hirabayashi, and J. Diehl (Genentech).

Appendix

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