Recombinant human granulocyte colony-stimulating factor therapy for patients with neutropenia and/or neutrophil dysfunction secondary to glycogen storage disease type 1b

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The purpose of this study was to evaluate the efficacy and toxicity of recombinant human granulocyte colony-stimulating factor (rhG-CSF) therapy in patients with neutropenia and/or neutrophil dysfunction secondary to glycogen storage disease (GSD) type 1b. Thirteen patients with neutropenia and/or neutrophil dysfunction secondary to GSD type 1b were treated with rhG-CSF. The effects of therapy on neutrophil numbers and in vitro neutrophil function and on bone marrow cellularity and morphology were studied. The clinical status of the patients and the occurrence of adverse events associated with rhG-CSF use were monitored. Use of rhG-CSF therapy was associated with a significant increase in circulating neutrophil numbers ($P < .01$) and an improvement in neutrophil function as assessed in vitro. In addition, rhG-CSF therapy produced a significant increase in marrow cellularity and an increase in myeloid:erythroid (M:E) ratio, indicating stimulation of granulopoeisis. No adverse effects on marrow function were noted; in particular, no myelodysplasia or marrow exhaustion was seen. Use of rhG-CSF therapy was associated with objective and subjective improvements in infection-related morbidity. The therapy was well tolerated, although all patients developed splenomegaly, and 5 patients developed mild hypersplenism that did not require any specific treatment. rhG-CSF therapy is efficacious in the management of neutropenia and neutrophil dysfunction associated with GSD type 1b. Patients on this therapy need to be monitored for hypersplenism. Continued follow-up will be necessary to confirm long-term safety; however, no significant short-term toxicity was noted. (Blood. 2001;97:376-382)

Introduction

Glycogen storage disease (GSD) type 1b results from a deficiency of the glucose-6-phosphate translocase enzyme. This enzyme transports glucose-6-phosphate into the lumen of the endoplasmic reticulum, where it is hydrolyzed by glucose-6-phosphatase into glucose and inorganic phosphate.[1] Absence of the translocase, therefore, results in an inability to liberate glucose from glucose-6-phosphate that may be derived from either glycogenolysis or gluconeogenesis. Consequently, patients with GSD type 1b are dependent on dietary carbohydrate to maintain euglycemia and are susceptible to fasting hypoglycemia and lactic acidosis. Hypoglycemia may cause coma and seizures; however, neurological damage is uncommon because the brain is protected by its ability to metabolize lactic acid. Other features of GSD type 1b include hepatomegaly, anemia, poor linear growth, and delayed pubertal development.[2] Anemia is of uncertain etiology, but it is likely to be multifactorial. Contributing factors could include chronic or recurrent infection; inflammatory bowel disease; and nutritional deficiencies of iron, vitamin B12, or folic acid. Poor growth and delayed development seem to be caused by an excess of counter-regulatory hormones, such as cortisol, that are secreted during chronic hypoglycemia.

In addition to the above features, patients with GSD type 1b frequently have neutropenia and/or neutrophil dysfunction and are consequently susceptible to recurrent infections. Infections most commonly involve the skin, perirectal area, ears, and urinary tract; however, severe or life-threatening infections, such as sepsis, pneumonia, and meningitis, may also occur. The most frequently isolated organisms include *Staphlococcus aureus*, group A streptococci, *Streptococcus pneumoniae, Escherichia coli*, and the *Pseudomonas* species.[3] The etiology of neutropenia and neutrophil dysfunction are not known; however, bone marrow aspirates performed on some GSD type 1b patients with neutropenia have revealed hypocellularity with a myelocyte:erythrocyte (M:E) ratio of less than 3:1 and maturation arrest beyond the myelocytic stage.[3] These findings suggest that at least in a proportion of the patients, impaired granulopoiesis may underlie the observed neutropenia. Defects in neutrophil chemotaxis and intracellular bacterial killing have also been reported in patients with GSD type 1b and probably contribute to the observed infection risk.[4-7] Defective intracellular bacterial killing is associated with diminished respiratory burst activity following phagocytosis.[8] These and other neutrophil function defects may be related in part to impaired calcium mobilization and diminished calcium stores, which result in impaired signaling in phagocytic cells.[9,10]

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) has in vitro activity which is identical to that of

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highly purified human G-CSF.[11] This activity includes support of growth of bone marrow–derived colony-forming unit granulocyte-macrophage (CFU-GM) in methylcellulose culture; enhanced neutrophil-mediated, antibody-dependent cellular cytotoxicity; and induction of the expression of chemotactic receptors on mature granulocytes. These observed activities suggest that rhG-CSF therapy may be beneficial for patients with GSD type 1b and neutropenia and/or neutrophil dysfunction. Clinically, administration of rhG-CSF has been associated with improved neutrophil numbers and a reduction in the severity and frequency of infection in patients with chronic severe neutropenia from a variety of causes.[12] Although there are case reports of G-CSF or GM-CSF use in patients with GSD type 1b,[10,13,14] cytokine therapy has not been adequately studied in this group of patients.

Because the etiology of the neutropenia and neutrophil dysfunction in patients with GSD type 1b is unknown and because cytokine therapy can be associated with both acute and late side effects, it is imperative to document that such therapy is beneficial before recommending routine use of cytokines in these patients. This is, to the best of our knowledge, the first time that cytokine therapy has been evaluated systematically in a relatively large group of patients with this disorder.

Patients, materials, and methods

The study, which was conducted in 3 stages, had a total duration of 3 years. During stage I (historical data period), careful history and review of clinical records were performed. Patients with a confirmed diagnosis of GSD type 1b were eligible for enrollment if they had a history of recurring infection and (1) severe neutropenia (defined as an absolute neutrophil count [ANC] $< 0.5 \times 10^9$ cells/L on at least 3 occasions for a minimum of one month) and/or (2) documented neutrophil dysfunction (as defined by any in vitro assay performed at each patient's local institution). Several of our patients had received treatment with G-CSF or GM-CSF prior to commencement of the trial. These patients were eligible for enrollment provided that historical data were available to confirm the presence of recurring infection and severe neutropenia and/or neutrophil dysfunction before the use of cytokine therapy.

During stage II (dose-equilibration period), patients were given an initial subcutaneous dose of 2.5 μg/kg/d rhG-CSF. If the mean ANC in the subsequent 2-week period was less than 1.0×10^9 cells/L, the dose was escalated to 5 μg/kg/d for the next 2 weeks. Subsequent dose escalations to 10, 20, and 30 μg/kg were permitted after 2 weeks of therapy at the previous dose level if the mean ANC remained at less than 1.0×10^9 cells/L. After reaching the highest dose level (30 μg/kg), therapy could be continued for up to 6 weeks in patients with an ANC remaining at less than 0.5×10^9 cells/L. Patients were considered treatment failures if after 6 weeks of therapy at the highest dose level, the ANC failed to increase more than 0.5×10^9 cells/L; rhG-CSF was then discontinued.

During stage III (maintenance period), patients received rhG-CSF at the lowest dose required to maintain a neutrophil count of more than 1.0×10^9 cells/L. During stage III, dose adjustments were permitted for patients who experienced a decline in their ANC $< 1.0 \times 10^9$ cells/L for 3 consecutive readings or who experienced an increase in their ANC $> 10.0 \times 10^9$ cells/L for 3 consecutive readings. Doses were escalated according to the schedule outlined in the dose-equilibration period for patients with a low ANC, and doses were decreased by 1 μg/kg per dose every second week for patients with an elevated ANC.

Bone marrow aspirates were performed prior to study entry, at 3 months after start of treatment, at one year after start of treatment, and annually thereafter. Bone marrow aspirates were assessed for cellularity, morphology, M:E ratio, and postmitotic:mitotic ratio. The postmitotic:mitotic ratio, computed as neutrophils + bands + metamyelocytes / myelocytes + promyelocytes + blasts, is reduced in patients with maturation arrest. This is therefore a useful test of the effect of therapy on granulopoeisis.[15] Marrow aspirates were sent for chromosome analysis at the same time points.

Neutrophil and monocyte function was assessed in vitro by measuring the production of oxygen radicals (superoxide anion [O_2^-] generation) in response to phorbol myristate acetate (PMA) and the chemotactic peptide f-methionine-leucine-phenylalanine (fMLF). We measured O_2^- generation as superoxide dismutase inhibitable cytochrome C reduction by a continuous recording spectrophotometer as described previously.[8,9,16] Patient cells were assayed in parallel with cells obtained from healthy volunteer donors. The mean activity of patient-derived cells was expressed as a percentage of the mean activity of healthy donor cells. Paired assays were run at study entry (less than 3 months from start of therapy) and at 6-12 months from start of therapy.

During the study patients were monitored for infection, antibiotic use, and hospitalization. In addition, patients were evaluated for the presence of oral ulcers, gingivitis, periodontal disease, otitis media, and skin infections. A complete physical examination, including assessment of liver and spleen size, was performed at each clinic visit. Patients were asked to maintain a diary to assess compliance and subjective response to therapy. The Research Ethics Board or Institutional Review Board of each of the participating institutions approved this study. Informed consent was obtained from all patients or their parents.

Statistical analysis

The prestudy ANC for each patient was defined as the mean of all available ANCs prior to initiation of rhG-CSF. The steady-state ANC was defined as the mean of all available ANCs during the maintenance phase of the study. Patients who were neutropenic prior to enrollment were considered to have had a (1) complete response (CR) if the steady-state ANC was more than 1.0×10^9 cells/L; (2) partial response (PR) if the steady-state ANC was between 0.8 and 1.0×10^9 cells/L, with at least a 100% increase over pretherapy ANC; (3) minor response (MR) if the mean ANC improved from less than 0.1×10^9 cells/L to more than 0.2×10^9 cells/L but less than 0.5×10^9 cells/L; and (4) no response (NR) if none of the above criteria were met. For the whole group of patients, the mean pretherapy ANC was compared with the mean steady-state ANC using a paired t test, and $P < .05$ was considered statistically significant.

Data concerning neutrophil and monocyte function were considered in aggregate. Correction of respiratory burst activity to normal levels (ie, 100% of control) was considered to be a CR. An improvement of more than 50% over baseline was considered to be a PR, whereas an improvement of less than 50% was considered NR. Data concerning antibiotic use and infection were obtained by history and were not necessarily complete; therefore it was not possible to quantify antibiotic use or to quantify the frequency or severity of infection in the prestudy and on-study periods. Consequently, statistical analysis of the effect of therapy on infection was not possible. To estimate clinical efficacy, we used patients who had a history of at least one hospital admission for intravenous (IV) antibiotic therapy to treat suspected or documented infection within the 12-month period prior to the start of any cytokine therapy. These patients were considered to have had an objective clinical response (OCR) if they were not hospitalized for treatment of suspected or documented infection during the 3-year study period. The patients were considered to have had no clinical response (NCR) if at least one hospital admission for treatment of suspected or documented infection occurred during the study period. In addition, patients were asked to subjectively rate the frequency and severity of oral ulceration, gingivitis, and oral antibiotic usage for non–life-threatening infections. Patients were considered to have had a response if their subjective evaluation indicated an improvement in these symptoms.

Results

Patient population

Between November 1993 and August 1995, a total of 13 patients were enrolled in the trial. Historical data gathered during study stage I are presented in Table 1. At study entry, the patients ranged in age from 8 months to 23 years and 10 months (mean, 9 years and 11 months). There were 10 females and 3 males. Twelve (92%) of the 13 patients had documented severe neutropenia (ANC $< 0.5 \times 10^9$ cells/L) prior to the start of therapy and were therefore evaluable for neutrophil responses. Chart review revealed that various neutrophil function assays had been performed on 6 (46%) of 13 patients prior to study enrollment, and all of these patients had neutrophil dysfunction. Abnormalities seen included impaired chemotaxis (n = 6) and impaired bacterial killing of *S aureus* (n = 4). Six patients had received some cytokine therapy prior to study enrollment, and 5 of these patients had received more than 3 months of treatment. Eleven (85%) of the 13 patients had a history of hospital admission for treatment of documented or suspected infection within the 12 months prior to the start of any cytokine therapy. These patients were evaluable for clinical response. All patients or their parents provided subjective evaluations of clinical status as outlined above.

rhG-CSF dosage

The mean starting rhG-CSF dose was 2.7 μg/kg/d, and the mean steady-state dose was 5.2 μg/kg/d, with a dose range of 3-7.5 μg/kg/d.

Neutrophil responses

ANC data are shown in Table 2. Twelve patients were evaluable for neutrophil responses. Eleven (92%) of these 12 patients had a CR as defined previously, and one patient (8%) had a PR. For the whole group, the mean pretherapy ANC = 0.46×10^9 cells/L, and the mean ANC during the maintenance phase was 2.43×10^9 cells/L ($P < .01$).

We have previously demonstrated markedly reduced oxygen radical production in response to a variety of different stimuli in both neutrophils and monocytes from GSD type 1b patients.[8-10,13,16] Neutrophils and monocytes were isolated from 11 of our patients at various times through the course of rhG-CSF therapy, and O_2^- generation was assessed in response to either PMA or fMLP stimulation. Of the 11 patients tested, only 2 (UPN 26 and UPN 33) did not demonstrate severe impairment of oxygen radical production prior to rhG-CSF therapy. Neutrophil and monocyte function data are shown in Table 3. Early in rhG-CSF therapy (0-3 months after start of rhG-CSF), O_2^- generation in response to either PMA or fMLP was significantly depressed in both neutrophils and monocytes from GSD type 1b patients compared with controls ($P < .01$; Table 3, group 1). Following 6-12 months of rhG-CSF therapy there was a selective improvement in O_2^- generation (Table 3, group 2). Neutrophil and monocyte responses to PMA increased in group 2 compared to group 1, but they were still significantly decreased compared to controls ($P < .05$). In contrast, when fMLP was used as stimuli, O_2^- generation was increased to 195% of controls in GSD type 1b neutrophils ($P < .05$) and corrected to near-normal levels in patient monocytes (Table 3, group 1). Thus, rhG-CSF therapy resulted in a selective improvement in O_2^- generation that was stimulus-dependent. This selective correction in neutrophil and monocyte dysfunction by rhG-CSF

Table 1. Historical data

UPN	Sex	Age at study entry (y, m)	Prior cytokine therapy	Severe neutropenia	Known neutrophil dysfunction	IV antibiotic therapy
21	F	5y 8m	yes	yes	yes	yes
22	M	8m	yes	N/A	yes	no
23	M	23y 10m	no	yes	yes	yes
24	F	9y 2m	no	yes	no	yes
25	F	8y 2m	yes	yes	yes	yes
26	F	8m	no	yes	no	yes
27	F	5y 0m	yes	yes	no	yes
28	F	10y 8m	yes	yes	no	yes
31	F	16y 1m	yes	yes	yes	yes
32	F	9y 10m	no	yes	yes	yes
33	F	6y 7m	no	yes	no	yes
34	F	20y 11m	no	yes	no	no
35	M	13y 1m	no	yes	no	yes

Historical data were obtained by review of patients' medical records. Prior cytokine therapy indicates any cytokine therapy given at any time prior to study enrollment. Severe neutropenia indicates ANC $< 0.5 \times 10^9$ cells/L on 3 separate occasions at least one month prior to initiation of cytokine therapy. Known neutrophil dysfunction indicates that the patient had at least one abnormality in neutrophil function which was documented by laboratory assessment prior to study enrollment. IV antibiotic therapy indicates any hospital admission for IV antibiotic therapy for proven or suspected infection within the 12 months prior to initiation of any cytokine therapy. N/A indicates not available.

Table 2. Neutrophil response

UPN	Pretherapy ANC, mean	Steady-state ANC, mean	Response
21	0.4	1.8	CR
22	N/A	2.7	N/A
23	1.3	5.13	N/A
24	0.27	1.44	CR
25	0.68	2.20	CR
26	0.13	1.83	CR
27	0.27	0.89	PR
28	0.64	2.15	CR
31	0.34	3.11	N/A
32	0.58	1.82	CR
33	0.30	2.22	CR
34	0.14	3.0	CR
35	0.44	3.36	CR
	mean = 0.46	mean = 2.43	$P < .01$

Pretherapy ANC indicates the mean of all available ANCs for each patient prior to initiation of cytokine therapy. The steady-state ANC indicates the mean of all available ANCs for each patient during maintenance phase. CR is the mean ANC $> 1.0 \times 10^9$ cells/L, and PR is the mean ANC between 0.8 and 1.0×10^9 cells/L. The *P* value was obtained by the *t* test for paired data.

Table 3. Effect of rhG-CSF therapy on neutrophil and monocyte O_2^- generation

Stimulus used for O_2^- generation	Group 1 0-3 months (n = 8)		Group 2 6-12 months (n = 8)	
	Neutrophils	Monocytes	Neutrophils	Monocytes
PMA, %	48 ± 13	30 ± 8	73.5 ± 10	67.5 ± 12
	$P < .01$	$P < .01$	$P < .03$ (PR)	$P < .04$ (PR)
fMLP, %	32 ± 19	48 ± 10	195 ± 40	104 ± 12
	$P < .01$ (n = 4)	$P < .01$	$P < .05$ (CR)	P = NS (CR)

We measured O_2^- generation stimulated by either PMA or fMLP in neutrophils and monocytes obtained from controls and GSD type 1b patients. Patient O_2^- generation is expressed as the percentage of control O_2^- generation (mean ± SEM). Patients in group 1 were tested within 3 months of starting cytokine therapy, and patients in group 2 were tested after 6-12 months of therapy. The *P* value is the difference between the mean activity for patient's cells and control cells, and $P < .05$ indicates significant difference. PR indicates that O_2^- generation is improved to more than 50% of control, and CR indicates that O_2^- generation is equivalent to or better than control. NS indicates not significant.

Table 4. Bone marrow morphology

	Cellularity		Granulopoiesis		PMN (%)		Post-mitotic:mitotic ratio		M:E ratio	
UPN	Pretherapy	On therapy	Pretherapy	On therapy	Pretherapy	On therapy	Pretherapy	On therapy	Pretherapy	On therapy
21	normal	hypercellular	arrest at myelocytes	normal	6	28	0.6:1	1:1	0.8:1	6:1
22	—	hypercellular	—	normal	—	23	—	0.7:1	—	6:1
23	hypercellular	hypercellular	normal	normal	44	—	7:1	3.6:1	5:1	9:1
24	hypercellular	hypercellular	normal	normal	8	13	2.4:1	1:1	5:1	8:1
25	hypercellular	hypercellular	arrest at bands	normal	—	35	—	1.8:1	—	6:1
26	normal	hypercellular	arrest at metamyelocytes	normal	5	23	1.9:1	1.6:1	1.5:1	4:1
27	hypercellular	normal	normal	normal	3	11	1.3:1	5.5:1	1.7:1	5.2:1
34	hypercellular	hypercellular	normal	normal	32	28	3:1	0.8:1	6:1	8:1
35	hypercellular	hypercellular	normal	normal	—	—	—	—	10:1	—

Bone marrow morphology was assessed on Wright-Giemsa stain. PMN indicates the percentage of segmented neutrophil identified on a differential count of total nucleated cells. The postmitotic:mitotic ratio equals the neutrophils + bands + metamyelocytes/myelocytes + promyelocytes + blasts.

therapy was unaltered by further treatment with rhG-CSF. One patient (UPN 31) was retested 5 years into rhG-CSF therapy and found to have reduced neutrophil O_2^- generation in response to PMA (48% of control, $P < .01$) and enhanced response to fMLP (161% of control, $P < .01$). The enhanced response to fMLP suggests that rhG-CSF therapy may have a priming effect on neutrophil function.

Changes in bone marrow morphology

Bone marrow aspirates were evaluated for morphology, cellularity, differential counts, postmitotic:mitotic ratio, and M:E ratio. We reviewed paired pretherapy and maintenance therapy bone marrow aspirates that were available for 9 patients. Results for these 9 patients are shown in Table 4. The cellularity was normal in 2 patients and increased in 7 of these 9 patients prior to therapy. Three patients showed maturation arrest in the granulocyte series at the band, metamyelocyte, or myelocyte stage and a marked paucity of mature forms during pretherapy, and 6 patients showed normal granulopoiesis with all stages of maturation during pretherapy. During maintenance therapy all 9 patients had normal granulopoiesis with all stages of maturation. Prior to therapy the segmented neutrophils expressed as a percentage of the bone marrow differential count was a mean of 16% (range, 3% to 44%). This increased to 25% (range, 11% to 35%) during the maintenance phase of therapy.

The mean pretherapy post-mitotic:mitotic ratio for our patients was 2.7:1 (range, 0.6:1 to 7:1). The post-mitotic:mitotic ratio did not change significantly during the maintenance phase of therapy (mean, 2:1; range, 0.7:1 to 5.5:1). The mean pretherapy M:E ratio for our patients was slightly elevated at 4.3:1 compared with the normal value of 3:1.[17] For each patient the M:E ratio increased during rhG-CSF therapy, and for the whole group the mean M:E ratio increased to 6.5:1. None of the patients showed changes consistent with a diagnosis of myelodysplasia or developed cytogenetic abnormalities in the marrow during therapy.

Effects of rhG-CSF on infection-related morbidity

Infection-related morbidity is summarized in Table 5. Of the 11 patients who had at least one hospitalization for IV antibiotics prior to study enrollment, 8 patients were not hospitalized during the study, and the objective CR rate was 73%. Two of the 3 patients with NCR had one hospital admission each for IV antibiotic therapy. One of these patients (UPN 25), who had a history of meningitis with brain abscess and recurrent pneumonia requiring frequent hospitalization prior to rhG-CSF therapy, was admitted on only one occasion, for treatment of suspected pneumonia, while on therapy. A second patient (UPN 35) was admitted for treatment of periodontal abscess, which occurred at a time when his neutrophil count had dropped to less than 0.5×10^9 cells/L during the maintenance phase of therapy. The remaining patient (UPN 31) had frequent hospitalizations for fever, which were usually associated with neutropenia. The neutropenia promptly resolved in hospital with supervised administration of rhG-CSF, which called this patient's compliance with therapy into question.

Eleven patients had a history of recurrent oral ulceration and/or gingivitis prior to rhG-CSF therapy. The 2 patients without a positive history were both 8 months of age at study entry. All patients with a positive history reported an improvement in the duration and frequency of oral ulceration, and 3 patients had a complete resolution, with no oral ulceration occurring during the study. All 13 patients had a history of recurrent oral antibiotic use for treatment of superficial infections, and all patients subjectively reported a decrease in the incidence and frequency of oral antibiotic usage. One patient (UPN 23), who had been diagnosed with Crohn's disease prior to therapy, experienced a resolution of symptoms. This is consistent with previous case reports.[18]

Table 5. Clinical response

	Objective response			Subjective response	
	IV antibiotic therapy				
UPN	Pretherapy	On therapy	Response	Oral ulceration	Oral antibiotic use
21	yes	no	OCR	improved	improved
22	no	no	N/A	N/A	N/A
23	yes	no	OCR	improved	improved
24	yes	no	OCR	improved	improved
25	yes	no	OCR	improved	improved
26	yes	no	OCR	N/A	N/A
27	yes	yes	NCR	improved	improved
28	yes	no	OCR	improved	improved
31	yes	yes	NCR	improved	improved
32	yes	no	OCR	improved	improved
33	no	no	N/A	improved	improved
34	yes	no	OCR	improved	improved
35	yes	yes	NCR	improved	improved

Subjective response indicates as self-reported by the patient. IV antibiotic therapy includes any hospital admission for IV antibiotic therapy for proven or suspected infection within the 12 months prior to initiation of any cytokine therapy. Pretherapy indicates the 12-month period before the start of any cytokine therapy; on therapy, the 3-year study period. OCR indicates objective clinical response (no hospitalization for IV antibiotics during the study), and NCR indicates no clinical response (at least one hospitalization for IV antibiotics occurred during the study). N/A = not applicable.

Table 6. Toxicity

UPN	Injection site reaction	Systemic symptoms	Splenomegaly	Hypersplenism	Lowest platelet count	Leukoerythroblastic response	Left shift	Other	Completed study
21	no	no	yes	no	—	no	yes	—	yes
22	no	no	yes	no	—	no	yes	widening of diploic spaces	yes
23	no	headache, muscle aches	yes	no	—	yes	yes	—	no
24	no	nausea	yes	no	—	yes	yes	—	yes
25	no	no	yes	no	—	no	yes	—	yes
26	no	no	yes	no	—	yes	yes	—	yes
27	no	no	yes	yes	114	no	yes	—	yes
28	no	no	yes	yes	92	no	no	—	yes
31	no	no	yes	yes	85	no	yes	splenectomy	yes
32	no	no	yes	yes	82	no	yes	—	yes
33	no	bone pain	yes	yes	74	no	yes	—	yes
34	no	no	yes	no	—	no	no	—	yes
35	no	no	yes	no	—	no	yes	—	yes

Toxicity was determined by review of patient diaries and case report forms. Hypersplenism was defined as a platelet count of less than 150×10^9 cells/L on 2 consecutive complete blood counts.

Toxicity

Adverse events experienced by the patients in this trial are summarized in Table 6. None of the patients experienced injection site reactions such as redness or swelling. Three patients had transient systemic symptoms including headache, muscle aches, nausea, and bone pain, all of which were mild and resolved with continued therapy. Although none of the patients had splenomegaly prior to therapy, all patients developed splenomegaly while on therapy, usually within 3 months of beginning therapy.

Splenomegaly was dramatic, with spleen tips palpable up to 13 cm below the costal margin (range, 3-13 cm). We defined hypersplenism as thrombocytopenia (platelet count less than 150×10^9 cells/L) present on at least 2 consecutive complete blood counts at least one month apart, in association with splenomegaly. Five patients developed hypersplenism by these criteria. Thrombocytopenia was mild, with the lowest recorded platelet counts ranging between 74 and 114×10^9 cells/L. Platelet counts tended to fluctuate during the course of therapy. One patient (UPN 31) had a splenectomy to treat chronic anemia that was presumed to be hemolytic in nature. This patient's lowest recorded platelet count was 85×10^9 cells/L, and this was recorded 16 months prior to splenectomy. At the time of splenectomy, the platelet count was normal at 225×10^9 cells/L. This patient's spleen showed sinusoidal hyperplasia with large numbers of neutrophils including some immature forms, but the spleen showed no other abnormality. These findings were interpreted as consistent with trapping of neutrophils in the spleen. The anemia did not resolve following splenectomy and was subsequently diagnosed as being secondary to vitamin B12 deficiency. No other patient required therapy to manage thrombocytopenia, and no patient had a bleeding complication.

Eleven (85%) of 13 patients experienced a marked left shift in circulating neutrophils at some point during the course of therapy. Three patients (23%) developed what could be described as a leukoerythroblastic response, with blast cells being detected in the circulation. In all 3 patients the leukoerythroblastic response was transient. In one patient, rhG-CSF was temporarily discontinued and then resumed at a lower dose following resolution of the leukoerythroblastic response.

One patient (UPN 22), who was 8 months old at the time of enrollment, was noted to have macrocephaly, with a head circumference just above the 97th percentile for age. Computed tomography and magnetic resonance image scanning of this patient's head revealed that widening of the diploic spaces, presumably due to myeloid hyperplasia, was the cause for this mild macrocephaly. This patient's maximum and steady-state rhG-CSF dose was 5 μg/kg/d, and no dosage adjustments were made because the macrocephaly was considered to be trivial.

Hemoglobin and hematocrit values for our patients are presented in Table 7. We defined anemia as either a hemoglobin or hematocrit below the age- and sex-appropriate reference range on at least 2 consecutive complete blood counts at least one month apart. By these criteria, 9 of our patients were anemic at some point during the study; however, 8 of these 9 patients were already anemic prior to study enrollment. For the whole group, the hemoglobin at study entry ranged from 77-130 g/L (mean, 105 g/L), and the lowest recorded hemoglobin during the study ranged

Table 7. Hemoglobin and hematocrit values

UPN	Hb at study entry (g/L)	Hb range during study (g/L)	Hct range during study (%)	Reference range	
				Hb (g/L)	Hct (%)
21	123	111-128	32.7-37.5	120-160	36-48
22	110	96-118	28.4-34.7	110-140	33-42
23	100	82-107	25.5-35.5	130-180	40-53
24	115	102-134	31.3-39.0	110-140	33-42
25	123	103-130	32.8-40.1	120-160	36-48
26	83	78-116	24.5-34.8	110-140	33-42
27	103	103-115	30.8-32.4	110-140	33-42
28	130	113-142	33.0-42.0	120-160	36-48
31	108	66-118	20.0-34.9	120-160	36-48
32	96	91-112	29.2-34.1	120-160	36-48
33	115	100-121	29.0-35.7	120-160	36-48
34	91	91-107	29.2-33.8	120-160	36-48
35	77	77-99	23-29.4	120-160	36-48

Hb at study entry indicates the hemoglobin concentration prior to initiation of rhG-CSF; Hb range, the lowest and highest hemoglobin recorded during the study; and Hct range, the lowest and highest hematocrit recorded during study. The reference range for both hemoglobin and hematocrit are based on patient sex and age at study entry.

from 66 to 113 g/L (mean, 86 g/L). Although these differences did not appear to be significant, we cannot exclude the possibility that the splenomegaly may have exacerbated the anemia in some of the patients. As was the case with thrombocytopenia, the anemia tended to fluctuate during the course of therapy. As mentioned previously, patient UPN 31 underwent splenectomy in part to treat anemia; however, the anemia persisted after the splenectomy.

Twelve (92%) of the 13 patients completed the study. One patient (UPN 23) withdrew after nearly 3 years because he was concerned about the risk of myelodysplasia and hypersplenism. The remaining 12 patients all chose to remain on the drug following completion of the study.

Discussion

GSD type 1b is associated with both neutropenia and neutrophil dysfunction, which predispose patients to recurrent and sometimes severe or life-threatening infection. Use of rhG-CSF can stimulate marrow granulopoiesis and increase neutrophil production, but in addition it can enhance neutrophil function both in vivo and in vitro. In this study we prospectively evaluated the efficacy and toxicity of rhG-CSF therapy in patients with GSD type 1b. Our results show that patients with GSD type 1b and neutropenia or neutrophil dysfunction experience significant increases in ANC and improvement in in vitro neutrophil function, as assessed by O_2^- generation in response to PMA and fMLP, during therapy with rhG-CSF.

The increased ANC results, in part, from increased marrow granulopoiesis, as indicated by an increase in marrow cellularity, M:E ratio, and mature granulocyte percentage noted on marrow differential counts following initiation of therapy. Interestingly, the cellularity and M:E ratios were already normal or perhaps mildly elevated in the majority of our patients prior to therapy, and the post-mitotic:mitotic ratio did not indicate a maturation arrest. These findings are in contrast to previous reports[5] and seem to suggest that in some patients with GSD type 1b, the neutropenia may not result from an underproduction by the bone marrow, but rather the neutropenia may result from a failure to release mature granulocytes into the blood stream or from failure to mobilize marginating granulocyte pools. rhG-CSF may increase ANC in these patients by affecting neutrophil trafficking.

The use of rhG-CSF therapy significantly enhanced phagocytic cell production of O_2^- in response to both fMLP and PMA. The response was stimulus-dependent; fMLP-triggered O_2^- generation was enhanced, whereas PMA-induced O_2^- generation was reduced compared with controls. Similar stimulus-dependent responses to rhG-CSF therapy have been reported previously, and several possible mechanisms have been proposed.[19-25] The rhG-CSF therapy may modulate signal transduction pathways linked to receptors rather than directly altering components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The enhanced response to fMLP, but not to PMA, a direct activator of protein kinase C, indicates an alteration in signal transduction preceding protein kinase C activation. Increased membrane phospholipase activity in response to receptor-mediated agonists is a possible target.[19,26] O_2^- production is of greater magnitude and duration in response to PMA compared with fMLP. Decreased O_2^- generation in response to PMA following rhG-CSF therapy may be the result of an overall desensitization of phagocytic cells to stimulation following maturation in the presence of rhG-CSF.[22] Alternatively, rhG-CSF therapy may increase the number of immature circulating neutrophils, which would generate a less than maximal response. In summary, rhG-CSF therapy for more than 6 months was able to correct defective O_2^- generation in both neutrophils and monocytes of GSD type 1b patients. The correction was stimulus-dependent, which suggests that rhG-CSF modulates receptor-linked signal transduction pathways and does not act directly on components of the NADPH oxidase.

Our study did not follow a prospective, randomized placebo-controlled design. In addition, we were not able to quantify the frequency or severity of infection or antibiotic use, and so comments on the clinical utility of rhG-CSF therapy for these patients need to be made with some caution. The majority of our patients did, however, experience both objective and subjective improvement in infection-related morbidity. In particular, the frequency of hospital admission for IV antibiotic therapy to treat suspected or documented infection declined significantly. In fact, only 2 patients had documented invasive bacterial infection while on the study. Both of these patients were neutropenic at the time of infection, which suggests that compliance with therapy and careful monitoring to maintain an ANC $> 1.0 \times 10^9$ cells/L may be critical to obtain optimum effectiveness. To be sure, many of the pretherapy admissions for IV antibiotics were possibly precautionary because of fever in the presence of neutropenia or known neutrophil dysfunction; however, the freedom from hospitalization following therapy still represents a significant improvement in status for these patients. In addition, all patients reported subjective improvements in frequency and severity of oral ulceration, gingivitis, superficial infection, and oral antibiotic usage. These data strongly suggest that rhG-CSF is clinically efficacious in reducing the incidence and severity of infection in patients with GSD type 1b. Such an improvement would not be unexpected based on the observed effects of therapy on neutrophil numbers and function, and would be consistent with results from previous case reports[16,17] in which the use of G-CSF or GM-CSF was associated with improvement in neutropenia and clinical status in patients with GSD type 1b.

The dose of rhG-CSF required by our patients was low (range, 3-7.5 μg/kg/d), and the medication was well-tolerated. There were no identified adverse effects of therapy on marrow function. In particular, myelodysplasia or marrow exhaustion was not encountered; however, it should be noted that this study comprised a small group of patients and a relatively short follow-up. Three patients who showed maturation arrest in granulopoiesis with a relative paucity of mature granulocytic forms experienced correction to normal morphology while on therapy.

Mild to moderate anemia was seen frequently in our patients, both prior to and after initiation of rhG-CSF. This is consistent with previous reports. For example, in a series of 5 adult patients with GSD type 1b, none of whom were receiving cytokine therapy, all 5 patients were anemic.[2] Although hemoglobin and hematocrit values were not provided for the GSD type 1b patients, in the same report, such data were provided for 32 adult patients with GSD type 1a; 26 (81%) of these 32 patients were anemic. For men the hemoglobin concentrations ranged from 64-144 g/L (average, 115 g/L), and hematocrits ranged from 22.2% to 43.3% (average, 35.1%). For women the hemoglobin concentrations ranged from 80-129 g/L (average, 108 g/L), and the hematocrits ranged from 27.4% to 39.8% (average, 32.8%). These values are similar to values observed in our patients prior to and during the study. Although we cannot be certain that hypersplenism did not contribute to the anemia, a direct effect of rhG-CSF seems unlikely. The Severe Chronic Neutropenia Registry has data on over 700 patients

who have received G-CSF for up to 12 years, and G-CSF therapy has not been noted to adversely affect hemoglobin levels (M. H. Freedman, director of the Severe Chronic Neutropenia Registry, oral communication, March 2000).

The splenomegaly we have observed is of some concern. This side effect of rhG-CSF therapy seems to be peculiar to patients with GSD type 1b, and its etiology is unclear. One of our patients underwent splenectomy, and histological evaluation of the spleen showed only sinusoidal hyperplasia with trapping of neutrophils. Thrombocytopenia associated with splenomegaly has been mild, not associated with any bleeding diathesis, and has generally tended to wax and wane during the course of therapy; therefore, unless thrombocytopenia was severe and persistent, we would not recommend splenectomy or dosage adjustment in those patients with splenomegaly. None of our patients developed Sweet syndrome or complications other than those mentioned previously.[27] In conclusion, our data confirm the short-term safety and efficacy of rhG-CSF therapy for GSD type 1b patients. However, continued monitoring and long-term follow-up will be required to ensure that this therapy has no significant late effects.

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