American Journal of Hematology 50:288–291 (1995)

Treatment of Chronic Neutropenia Associated With Large Granular Lymphocytosis With Cyclosporine A and Filgrastim

Ann Jakubowski, Elliott F. Winton, Alison Gencarelli, and Janice Gabrilove

Department of Medicine and Nursing, Memorial Sloan-Kettering Cancer Center, New York, New York (A.J., A.G., J.G.), and Department of Medicine, Emory University School of Medicine, Atlanta, Georgia (E.F.W.)

A patient with neutropenia and life-threatening infections secondary to T-γ lymphoproliferative disease, who did not respond to treatment with recombinant human G-CSF (filgrastim), was treated with filgrastim plus cyclosporine A (CyA). The patient achieved a good response in the absolute neutrophil count and subsequently required a dose reduction in the filgrastim. The patient was eventually discontinued from the CyA but continues on filgrastim alone. While on therapy, the large granular lymphocytes disappeared from the circulation and the beta-TCR rearrangement, which was present prior to beginning therapy, became undetectable. The patient had no significant toxicity to the CyA or the filgrastim and he has not experienced any serious infections or required hospitalization. Filgrastim has proven to be relatively nontoxic and of some benefit to patients with this disease and should probably be utilized first when treatment is necessary. However, if improvement is not observed, these findings suggest that a trial of the combination of CyA plus filgrastim may be beneficial. © 1995 Wiley-Liss, Inc.

Key words: granulocyte colony stimulating factor (filgrastim), lymphoproliferative disorders, cyclosporine A (CyA)

INTRODUCTION

The large granular lymphocyte syndrome, associated with the proliferation of T-lymphocytes, frequently manifests itself with the sequelae of neutropenia. Although generally an indolent disorder, treatment is precipitated by recurrent serious infections. Therapy for these patients has included corticosteroids, splenectomy, and more recently, hematopoietic growth factors, all with limited success. This report describes a patient whose neutrophil count did not respond to filgrastim alone but did normalize when the immunomodulator cyclosproine A was added.

In 1977, a syndrome characterized by the proliferation of larger than normal lymphoid cells with abundant cytoplasm and azurophilic granules was first described [1]. Recently Loughran reviewed the literature on the clonal diseases of large lymphocytes [2]. Evidence has accumulated that these large granular lymphocyte (LGL) disorders may originate from either T-lymphocytes or natural killer (NK) cells [3,4]. Cytogenetic abnormalities and T-cell receptor gene rearrangements have confirmed their clonal nature [5,6]. The T-LGL proliferations follow a more chronic clinical course than the NK type [2]. Frequently, they are associated with chronic neutropenia which may be severe, autoimmune features which include arthritis, and recurrent infections. Anemia and thrombocytopenia occur less frequently. A "maturation arrest" of the myeloid series and lymphocytic infiltration is observed in the bone marrow of most patients [2]. Some studies have demonstrated antineutrophil antibodies and decreased neutrophil survival, but the etiology of the neutropenia is not clearly understood [7]. Although many patients are stable clinically for extended periods of time, a proportion eventually require therapy for cytopenias and associated recurrent bacterial infections [8–10]. Corticosteroids and splenectomy have been of limited usefulness and recently granulocyte and granulocyte-macro-

Received for publication March 22, 1995; accepted June 14, 1995.

Address reprint requests to Ann Jakubowski, M.D., Ph.D., Memorial Sloan-Kettering Cancer Center, 1275 York Ave., New York, NY 10021.

phage colony stimulating factor (G- and GM-CSF) have been utilized as treatment in a small number of patients [8,11–14]. G-CSF has been used most frequently but efficacy of both has been limited. We report on a patient treated with recombinant human G-CSF (filgrastim) plus cyclosporine A (CyA), who previously had not responded to filgrastim alone.

CASE REPORT

The patient is a 51-year-old businessman who developed a pseudomonas cellulitis of the right foot associated with severe neutropenia and thrombocytopenia while vacationing in Australia. He recovered with antibiotics and returned to the United States for further workup.

On initial evaluation elsewhere, the laboratory studies were suggestive of a T-lymphoproliferative process. The patient was treated with cyclophosphamide 50 mg and prednisone 50 mg daily, but without improvement. He was referred for a second opinion.

Evaluation at this institution included a physical examination remarkable only for mild folliculitis over the chest and scalp; white blood cell count = 6,100 cell/μl; absolute neutrophil count (ANC) = 180 cells/μl; absolute lymphocyte count (ALC) = 3,170 cells/μl; hemoglobin = 15.1 g/dl; and platelet count = 419,000 cells/μl. The peripheral blood smear exhibited a predominance of LGLs. The surface phenotype of that population was CD2+(95%), CD3+(91%), CD4+(40%), CD5+(57%), CD7+(62%), CD8+(53%), and Southern blot analysis demonstrated a T-cell receptor gene rearrangement of the beta segment (beta-TCR); all consistent with a CD3+ T-cell granular lymphoproliferative disorder. A bone marrow biopsy was hypocellular and the aspirate demonstrated a paucity of myeloid precursors. Megakaryocytes were normal and eosinophils were increased. Rheumatoid factor was weakly positive, anti-nuclear and antineutrophil antibodies were undetectable. Cyclophosphamide and prednisone were discontinued.

With informed consent, the patient was subsequently given a trial of filgrastim 1–20 mcg/kg/d with minor improvement in the ANC. Similarly, a course of pentoxyphylline was given without success. During treatment of a staphylococcal bacteremia, filgrastim 20 mcg/kg/d was used in addition to the antibiotics. The ANC never exceeded 1,000 cells/μl but the folliculitis improved. Finally, in the fall of 1991, CyA 4 mg/kg/d (150 mg po BID) was added to the 20 mcg/kg/d of filgrastim with a good response in the ANC.

Peripheral blood counts were used to follow the efficacy of each change in therapy, which successfully produced an ANC >1,000 cells/μl (Fig. 1). As the ANC began to recover, the filgrastim dose was reduced, but could not be discontinued (d439–444). Subsequently the CyA dose was reduced and eventually discontinued. The

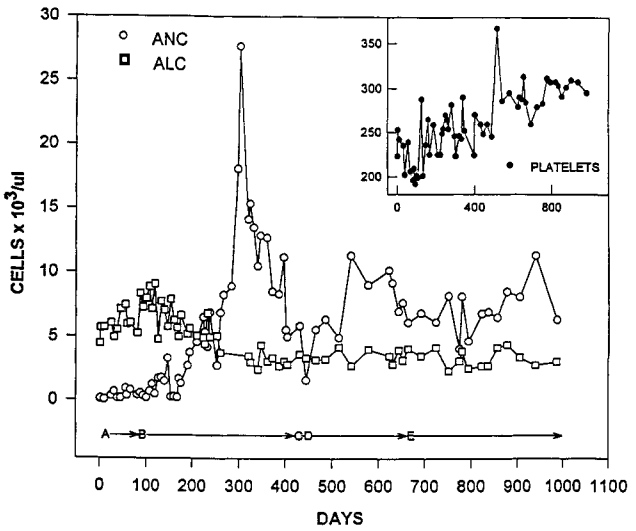

Fig. 1. Peripheral blood count changes while on therapy. Treatment: A → B (day 1–80), filgrastim 20 mcg/kg/d; B → C (day 81–438), dose adjusting filgrastim to 0.5 mc/kg/d, adding CyA, adjusting dose to 300 mg; C → D (day 439–443), filgrastim discontinued, CyA 300 mg; D → E (day 444–652), 0.5 μg/kg/d restarted, CyA 300 mg; E → (day 653–present), filgrastim 0.5 mcg/kg/d, CyA discontinued. ANC: absolute neutrophil count; ALC: absolute lymphocyte count.

patient continues on 0.5 mcg/kg/d of filgrastim alone with a normal ANC. The ALC slowly decreased, and the LGLs disappeared from the circulation. The beta-TCR rearrangement became undetectable. The platelet count, which at baseline was at the upper limits of normal, remained within the normal range.

Bone marrow studies are summarized in Table I. The hypocellular biopsies increased in cellularity only with the combination of CyA plus filgrastim. This was due primarily to a repopulation of the myeloid lineage. The numbers of eosinophils decreased.

Clinically, the patient had no significant toxicity to the CyA or the filgrastim, and he has not experienced any serious infections or required hospitalization.

DISCUSSION

Recently, immune-mediated mechanisms have been implicated in hematopoiesis and the pathophysiologic processes responsible for hematologic disorders such as aplastic anemia [15]. Lymphocytes and monocytes, the major components of the immune response, function through the production of positive and negative regulators of hematopoiesis. The immunosuppressive agent CyA is believed to inhibit the production of interleukin-2 (IL-2) and gamma-interferon (g-IFN) by T-lymphocytes, as well as the activation of cytotoxic T-cells by IL- 2 [16]. Both IL-2 and g-IFN have been shown in vitro and/or in vivo

TABLE I. Laboratory Data*

	Date	8/6/90	5/6/91	6/17/91	3/9/92	6/29/92	5/19/93	6/13/94
Therapy		None	G 10	Pentoxy 400	G 20 CyA 200	G 2 CyA 300	G 0.5 CyA 300	G 0.5
Biopsy	Cellularity	20–30%	ND	ND	ND	70–80%	ND	50%[a]
Aspirate differential	Myeloid	18	31	ND	30	61	53	53
	Erythroid	53	34		37	10	12	25
	Lymphoid	24	29		27	24	32	14
	M:E	0.3	0.9		0.8	6.1	4.4	2.1
	Neu:Eos	0	0.04		0.1	3.5	16.3	7.5
DNA studies	IgH	Germline	ND	ND	ND	ND	Germline	Germline
	β-TCR	Rearranged					Germline	Germline
Peripheral blood	WBC[b]	6.1	8.6	11.1	10.5	19.8	13.3	10.6
	ANC[b]	0.18	0.09	0.4	3.8	15.4	9.2	6.4
	ALC[b]	5.2	7.8	10.1	5.7	3	2.8	3
	PLT[b]	419	237	306	259	240	290	295

*G, filgrastim [mcg/kg/day]; CyA, cyclosporine A [mg/d]; Pentoxy, pentoxyfilline [mg BID]; Neu:Eos, % neutrophils:% eosinophils; IgH, heavy chain gene rearrangement; beta-TCR, T-cell receptor gene (beta-segment) rearrangement; ND, not done.
[a]Patchy specimen.
[b]$\times 10^3$ cells/μl.

to inhibit the growth of hematopoietic progenitors and have been implicated in the development of aplastic anemia and red cell aplasia [17].

The clinical presentation of the T-cell LGL disorders and occasional responses to corticosteroids, splenectomy, and cytotoxic chemotherapy suggested a T-cell mediated immune mechanism directed at the neutrophil granulocyte lineage. Several studies had demonstrated improvement in neutrophil counts and clinical benefit from the use of filgrastim alone in other chronic severe neutropenic disorders, as well as occasional patients with LGL proliferation [8]. Escalating doses of filgrastim were utilized in this patient in order to try and establish a non-toxic therapy. Unlike the patients in previous reports, however, even doses of 20 mcg/kg/d produced no significant change in the peripheral blood counts or the bone marrow of this patient. Pentoxyfylline, an inhibitor of the effects of tumor necrosis factor, another suppressant of hematopoiesis, was also utilized without benefit.

Although spontaneous remission, which has been reported occasionally in these patients [18], cannot be completely ruled out, the success of adding CyA to filgrastim in our patient suggests the involvement of IL-2, g-IFN, or other inhibitor of hematopoiesis in the pathophysiology of this disorder. Furthermore, this mimics somewhat the results reported previously in two patients with aplastic anemia [19]. In several of the patients who responded to filgrastim alone, the bone marrow was hypercellular with a maturation arrest at the promyelocyte stage. The bone marrow of this patient was hypocellular due predominantly to decreased myeloid elements. This suggests inhibition at an earlier stage of myeloid development and perhaps another variant of the disease. The disappearance of the LGL from the circulation and the inability to detect the T-cell receptor gene rearrangement at the time of myeloid recovery also suggests a suppressive or cytotoxic effect on the clonal population of lymphocytes.

Filgrastim has proven to be relatively nontoxic and should be considered when treatment is necessary. If no improvement is observed, a trial of CyA plus filgrastim may be beneficial.

REFERENCES

1. McKenna RW, Parkin J, Kersey JH, Gajl-peczalska KJ, Peterson L, Brunning RD: Chronic lymphoproliferative disorder with unusual clinical morphologic ultrastructural and membrane surface marker characteristics. Am J Med 62:588, 1977.
2. Loughran TP: Clonal diseases of large granular lymphocytes. Blood 82:1, 1993.
3. Palutke M, Eisenberg L, Kaplan J, Hussain M, Kithier K, Tabaczka P, Mirchandani I, Tenenbaum D: Natural killer and suppressor T-cell chronic lymphocytic leukemia. Blood 62:627, 1983.
4. Loughran TP Jr, Kadin ME, Starkbaum G, Abrowitz JL, Clark EA, Disteche C, Lum LG, Slichter SJ: Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. Ann Intern Med 102:169, 1985.
5. Berliner N, Duby AD, Linch DC, Murre C, Quertermous T, Knott LJ, Azin T, Newland AC, Lewis DL, Galvin MC, Seidman JG: T cell receptor gene rearrangements define a monoclonal T cell proliferation in patients with T cell lymphocytosis and cytopenia. Blood 67:914, 1986.
6. Loughran TP, Starkebaum G: Large granular lymphocyte leukemia. Medicine 66:397, 1987.
7. Rustagi PK, Han T, Ziolkowski L, Farolino DL, Currie MS, Logue GL: Granulocyte antibodies in leukemic chronic lymphoproliferative disorders. Brit J Haematol 66:461, 1987.
8. Cooper DL, Henderson-Bakas M, Berliner N: Lymphoproliferative disorder of granular lymphocytes associated with severe neutropenia. Cancer 72:1607, 1993.
9. Garipidou V, Tsatalas C, Sinacos Z: Severe neutropenia in a patient with large granular lymphocytosis: prolonged successful control with cyclosporin A. Haematologica 76:424, 1991.

10. Loughran TP Jr, Starkebaum G: Large granular lymphocyte leukemia: report of 38 cases and review of the literature. Medicine 66:397, 1987.
11. Boxer LA, Hutchinson R, Emerson S: Recombinant human granulocyte colony-stimulating factor in the treatment of patients with neutropenia. Clin Immunol Immunopathol 62(Suppl):39, 1992.
12. Walls J, Dessypris EN, Kranz SB: Granulocyte colony-stimulating factor overcomes severe neutropenia of large granular lymphocytosis. Am J Med Sci 304:363, 1992.
13. Kaneko T, Ogawa Y, Hirata Y, Hoshino S, Takahashi M, Oshimi K, Mizogichi H: Agranulocytosis associated with granular lymphocyte leukemia: improvement of peripheral blood granulocyte count with human recombinant granulocyte colony-stimulating factor (G-CSF). Brit J Haematol 74:121, 1990.
14. Lang DF, Rosenfeld CS, Diamond HS, Shadduck RK, Zeigler ZR: Successful treatment of T-γ-lymphoproliferative disease with human-recombinant granulocyte colony-stimulating factor. Am J Hematol 40:66, 1992.
15. Loughran TP, Storb R: Treatment of aplastic anemia. BMT 4:559, 1990.
16. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB: Cyclosporin: a new immunosuppressive agent for organ transplantation. Ann Intern Med 101:667, 1984.
17. Zoumbos N, Gascon P, Djeu J, Young N: Interferon is the mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. Proc Natl Acad Sci USA 82:188, 1985.
18. Winton EF, Chan WC, Check I, Colenda KW, Bongiovanni KF, Waldmann WA: Spontaneous regression of a monoclonal proliferation of large granular lymphocytes associated with reversal of anemia and neutropenia. Blood 67:1427, 1986.
19. Kojima S, Fukuda M, Miyajima Y, Matsuyama T: Cyclosporine and recombinant granulocyte colony-stimulating factor in severe aplastic anemia. N Eng J Med 323:920, 1990.