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Successful Treatment of Refractory Anemia by High-Dose Methylprednisolone Associated With an Increment in CD68-Positive Cells in Bone Marrow

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Refractory anemia has a relatively low incidence of the subsequent development of acute leukemia and a relatively long survival among the myelodysplastic syndromes (MDS). We observed hematological improvement due to high-dose methylprednisolone in 9 of 18 patients with refractory anemia. The patients' age range was from 28 to 78 years old (mean age: 54), including 14 male and 4 females. A complete response was obtained in 5 patients, minimal response in 4 patients, and no response in 9 patients. Laboratory data of peripheral blood counts and differential counts of bone marrow aspirates were not different, except that fewer chromosomal abnormalities ($P = 0.086$) were observed in the responder group. Side effects were seen in two patients but were controllable. Overall survival was significantly longer in the responder group (Log-rank $P = 0.040$, Wilcoxon $P = 0.045$). The overall survival of responders did not reach the median and 85% of the patients were alive after 180 months, while the median overall survival of the non-responders was 61.8 months. Disease progression was more frequently seen in the non-responder group ($P = 0.045$). Furthermore, we investigated retrospectively immunohistochemical bone marrow staining, and a significantly higher percentage of CD68-positive (22.6% ± 7.1%) and CD45RA-positive cells was observed in the responder group compared to the non-responder group (6.5% ± 1.3%). Our present results indicate that high-dose methylprednisolone is valuable as a primary treatment before other immunosuppressive treatments, because of its ease of use. High efficacy with high-dose methylprednisolone is expected, especially in patients in which increments in CD68-positive cells in bone marrow are observed. Am. J. Hematol. 66:80–84, 2001.

Key words: methylprednisolone; refractory anemia; CD68; monocyte; macrophage; myelodysplastic syndrome

INTRODUCTION

Refractory anemia is associated with a relatively low incidence of the subsequent development of acute leukemia and a relatively long survival among the myelodysplastic syndromes (MDS) [1,2]. Although there are many therapeutic agents such as anabolic steroids (androgen), glucocorticosteroids, growth factors [3] (granulocyte colony-stimulating factor (CSF), granulocyte-macrophage CSF, erythropoietin, interleukin-3), differentiation-inducing agents (activated vitamin D3 [4], retinoid [4,5]), and cytotoxic agents (cytarabine [6] and cytarabine ocfosfate [7]), the effectiveness of treatment is usually low and a persistent effect without continuing medication is rare. The current treatment results for refractory anemia are unsatisfactory, and a more effective treatment is needed. We previously reported high-dose methylprednisolone therapy was effective in 2 of 5 patients with refractory anemia [8]. Since then in our hospital, we have been treating refractory anemia patients with high-dose methylprednisolone. In this paper, we de-

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scribe the hematological improvement due to high-dose methylprednisolone in 9 of 18 patients with refractory anemia. Furthermore, we investigated whether it is possible to predict responsiveness to high-dose methylprednisolone from clinical characteristics and immunohistochemical staining of bone marrow.

MATERIALS AND METHODS

Patients

Eighteen patients who were diagnosed with refractory anemia in our hospital from 1984 to 1996 were treated with high-dose methylprednisolone. Diagnoses were made according to the French-American-British classification [9] using bone marrow and peripheral blood specimens containing obvious bilineage or trilineage dysplasia. Ferrokinetics and chromosomal analysis were also performed to confirm diagnosis. No patient had a history of malignancy previously treated with chemotherapy. Methylprednisolone at 1,000 mg was administrated by drip infusion for 3 days under informed consent. When a response was not observed after 3 months of treatment, one more courses of methylprednisolone were given under informed consent. A final assessment was made 6 months after treatment.

Histological Preparation and Analysis

Bone marrow trephine biopsies were obtained at presentation from 18 patients with refractory anemia. In six patients from whom bone marrow biopsy specimens were not available, clot sections of bone marrow aspiration samples were used. Bone marrow biopsy or aspiration were done from the posterior superior iliac spine, fixed in 10% phosphate-buffered formalin, decalcified in neural EDTA for 24–72 hr, except for bone marrow aspiration samples, and then dehydrated in alcohol and embedded in paraffin. Sections were routinely stained with hematoxylin and eosin, and Giemsa.

Immunohistochemical Analysis

All paraffin sections were deparaffinized in xylene, dehydrated through a graded alcohol series to Tris buffer saline (TBS). The immunohistochemical staining used was a modification of a method already described [10]. Endogenous peroxidase was blocked by incubation for 30 min in methanol with 0.5% H_2O_2. Sections were washed in TBS for 5 min and then blocked in 5% skim milk for 10 min. All monoclonal antibodies were purchased from Dako Co. Ltd (Denmark). CD45RO (UCHL1: part of the leukocyte common antigen, memory T cell, myelomonocytic cell), CD45RA (part of the leukocyte common antigen, native T cell), leukocyte common antigen (LCA: lymphoid cells), CD68 (KP1: monocyte, macrophage, mast cell), CD3 (T cell), and L26 (B cell) were used as first antibodies and were applied with overnight incubation at 4°C. After being washed in TBS, a biotinylated goat antimouse immunoglobulin was applied and incubated for 60 min at room temperature. Peroxidase-conjugated streptavidin was then added and incubated for 60 min. The color was developed by using 3,3′- diaminobenzidine in 0.05 mol/l Tris buffer, pH 7.6, and nuclei were counter-stained with Mayer's hematoxylin. Normal sera were substituted for first antibodies as negative controls. One thousand nucleoited cells were counted, and the percentage of positive cells was analyzed.

Response Criteria

In all patients, more than 8 weeks of transfusion independence was required to obtain a hematological response. Responders were further categorized using the following criteria: complete response (CR), which featured almost normalization of all affected cell lines (hemoglobin, number of WBC, and platelets) and <5% marrow blasts present [11]; partial response (PR), characterized by $\geqq$50% improvement from baseline to normal levels of all affected cell lines and <5% marrow blasts present; minimal response (MR), noted by $\geqq$2 g/dl increase in hemoglobin or $\geqq 0.5 \times 10^9$/l increase in neutrophils or $\geqq 30 \times 10^9$/l increase in platelets.

Statistical Analysis

The correlation between immunohistochemical staining and responses to treatment, or to the laboratory data, was examined by Chi-square test. Overall survival was estimated by the Kaplan–Meier method, and comparisons were based on log-rank and Wilcoxon's tests.

RESULTS

Clinical Features

The patients' age range was from 28 to 78 years old (mean age: 54), including 14 males and 4 females. Fourteen patients were treated with one course of high-dose methylprednisolone, and 4 patients were treated with two courses of high-dose methylprednisolone. A complete response with high-dose methylprednisolone was obtained in 5 patients, a minimal response in 4 patients, and no response in 9 patients. Table I shows the peripheral blood counts of responders before and after high-dose methylprednisolone treatment. As listed in Table II, laboratory data of peripheral blood counts and differential counts of bone marrow aspirates were no different between the responders and non-responders. Four of 9 patients showed hypoplastic marrow in the responder group, and 6 of 9 patients showed hypoplastic marrow in the non-responder group, but there was no significant difference. Chromosomal abnormalities were observed in 9 patients, and the most frequent abnormalities were +8, −7, and 20q−. There was a tendency for chromosomal abnormali-

TABLE I. Changes in Peripheral Blood Counts Before and After High-Dose Methylprednisolone Treatment

No.	Age (years)	Sex	Hb (g/dl) Pre	Hb (g/dl) Post	WBC ($\times 10^9$/l) Pre	WBC ($\times 10^9$/l) Post	Plt ($\times 10^9$/l) Pre	Plt ($\times 10^9$/l) Post	Response
1	36	M	8.2	12.5	2.0	6.5	10	120	CR
2	27	F	8.8	12.0	2.1	4.5	80	150	CR
3	75	M	8.7	11.5	4.1	5.6	25	134	CR
4	51	M	6.7	15.0	5.6	8.2	41	198	CR
5	44	M	10.0	12.9	2.7	4.8	134	146	CR
6	78	F	6.2	10.1	2.1	2.8	9	21	MR
7	78	M	10.6	11.2	2.9	2.9	55	86	MR
8	74	M	11.1	13.2	4.5	5.4	77	58	MR
9	28	M	9.6	11.5	3.1	2.6	117	154	MR

TABLE II. Patient Characteristics Between Responders and Non-Responders

	Responders	Non-responders	*P*
Patient number	9	9	
Age	54.3	58.4	NS
Sex			
Male	7	7	
Female	2	2	NS
Hb (g/dl)	8.9	8.5	NS
WBC ($\times 10^9$/l)	35.0	28.0	NS
Granulocyte ($\times 10^9$/l)	17.0	12.0	NS
Lymphocyte ($\times 10^9$/l)	17.0	21.0	NS
Monocyte ($\times 10^9$/l)	3.5	1.5	NS
Platelet ($\times 10^9$/l)	82.0	57.0	NS
Reticulocyte (%)	1.5	1.6	NS
Bone marrow			
Nuclear cell count ($\times 10^9$/l)	94.0	71.0	NS
Megakaryocyte count ($\times 10^6$/l)	13	32	NS
Blast (%)	1.4	1.3	NS
Lymphocyte (%)	21.6	24.5	NS
Monocyte (%)	0.9	2.4	NS
Cellulality			
Hyper	1	0	
Normo	4	3	
Hypo	4	6	NS
Chromosomal abnormality	3	6	0.086
Progression	1	4	0.045

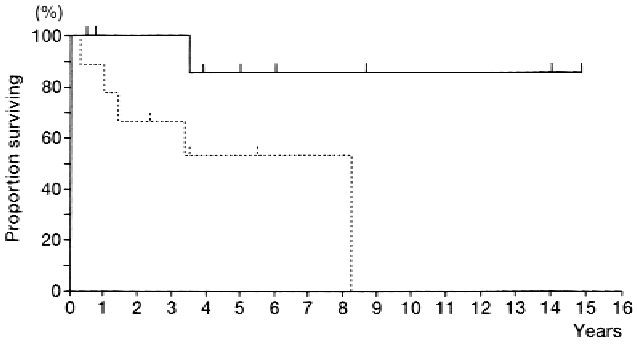

Fig. 1. Overall survival of refractory anemia patients treated with high-dose methylprednisolone (— responders and .... non-responders) (Log-rank *P* = 0.040, Wilcoxon *P* = 0.045).

TABLE III. Results of Immunohistochemical Staining

	Percentage of positive cells		
	Responders	Non-responders	*P*
UCHL1	6.5 ± 1.7	4.5 ± 1.3	NS
CD45RA	0.6 ± 0.2	0.2 ± 0.1	0.027
LCA	5.1 ± 1.1	3.4 ± 0.8	NS
KP1	22.6 ± 7.1	6.5 ± 1.3	0.041
CD3	3.9 ± 2.9	0.4 ± 0.3	NS
L26	0.5 ± 0.2	0.1 ± 0.2	NS

ties to be more frequent in the non-responder group (6/9) rather than the responder group (3/9) ($P = 0.086$). According to the international scoring system [12], there was a high percentage of patients who showed a low score in responder group, but this was not significant.

Long-Term Prognosis

Side effects were seen in two patients. One patient developed diabetes mellitus, and another patient developed activation of old pulmonary tuberculosis, but these patients were treated appropriately. Overall survival was significantly longer in the responder group (Log-rank $P = 0.040$, Wilcoxon $P = 0.045$) (Fig. 1). The overall survival of responders did not reach the median, and 85% of the patients were alive after 180 months; the median overall survival of non-responders was 61.8 months ($n = 9$). In the non-responder group, disease progression to refractory anemia with excess of blasts occurred in 3 cases, chronic myelomonocytic leukemia in 1 case, and acute myelogenous leukemia in 1 case. In the responder group, only one patient developed refractory anemia with excess of blast. The frequency of disease progression in the responder group was significantly lower than that in non-responders ($P = 0.045$).

Immunohistochemical Analysis

As shown in Table III, the mean percentage of CD68-positive cells in the responder group (22.6% ± 7.1%) was

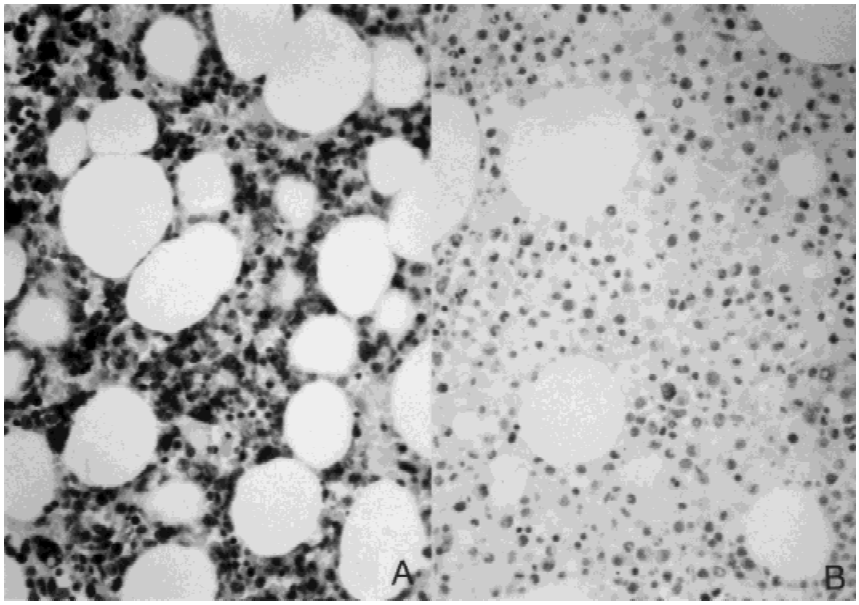

Fig. 2. Immunohistochemical staining of CD68-positive cells in bone marrow in responders (A) and non-responders (B) (original magnification ×400).

significantly higher than that (6.5% ± 1.3%) in the non-responder group. Figure 2 shows a typical immunohistochemical staining of CD68-positive cells from bone marrow in responders (A) and non-responders (B). The mean percentage of CD45RA-positive cells in the responder group was also higher than that in the non-responder group, although the percentage of CD45RA-positive cells was low.

DISCUSSION

In the present study, we demonstrated that high-dose methylprednisolone therapy was effective in 9 cases out of 18 patients with refractory anemia. We confirmed the effectiveness of high-dose methylprednisolone from our previous study [8] in a large number of patients. Many investigators have described immunological abnormalities in myelodysplastic syndrome such as arthritis, vasculitis, and serological features usually associated with autoimmune disease, but without clinical manifestations of autoimmunity [13–15]. Accordingly, we speculate that an immune mechanism may be associated with the etiology of myelodysplastic syndrome. Therefore, immunosuppressive drugs can be effective in some populations of refractory anemia patients. Recently, the effectiveness of cyclosporin A and antithymocyte globulin were reported in hypoplastic MDS patients [16]. Furthermore Jonasova et al. reported 14 (82%) of 17 patients with refractory anemia obtained a hematological response with cyclosporin A [17]. Molldrem et al. also reported antithymocyte globulin was effective in 9 (64%) of 14 patients with refractory anemia, although side effects such as anaphylaxis and serum disease developed in some patients [11]. These reports also suggest that immunosuppressive agents are an effective treatment in refractory anemia. An advantage of high-dose methylprednisolone therapy is that it requires only a short period of administration, improves the physical condition of patients, and serious side effects are relatively minor compared to antithymocyte globulin or cyclosporin A. In addition to this, the cost for the treatment is relatively low. In our study side effects occurred in 2 patients, but these problems were treated appropriately. When we take into consideration these merits, high-dose methylprednisolone therapy seems to be better than other more aggressive immune suppressive agents (antithymocyte globulin or cyclosporin A) as a primary treatment.

In order to clarify the difference between responders and non-responders taking high-dose methylprednisolone, we compared the clinical data and bone marrow immunohistochemical staining. A higher population of CD68-positive and CD45RA-positive cells was observed in the responder group than in the non-responder group. These results indicate that the cell population of macrophages, monocytes, mast cells, and native T cells in bone marrow cells increased in responders. Bone marrow macrophages have been reported to express a wide range of substances that may play complex regulatory roles in hematopoiesis [18]. Kitagawa et al. reported that overexpression of tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) were observed in 79% (11 out of 14) and 42% (5 out of 12) of bone marrow samples of myelodysplastic syndrome patients, respectively, using frozen bone marrow biopsy specimens [19]. They also observed the majority of these positive cells were CD68-positive macrophages. It was reported by Verhaef et al. [20] that the increase in serum TNF-α observed in MDS patients [21] was inversely correlated with low levels of

hemoglobin concentration. TNF-α and IFN-γ are well known as inhibiting factors of hematopoiesis [22]. Accordingly, one may hypothesize that the increase in bone marrow CD68-positive cells in refractory anemia patients is inhibited by high-dose methylprednisolone, thereby leading to hematological improvement. Our present data suggest that the effectiveness of high-dose methylprednisolone may be predicted in patients with increased CD68-positive cells in their bone marrow. Indeed, it has been reported that serum TNF-α decreased with high-dose methylprednisolone treatment in acute myelogenous leukemia and acute lymphoblastic leukemia 3 days after the initiation of therapy [23]. CD45RA-positive cells were higher in the responder group than the non-responder group. CD45RA expressed part of the leukocyte common antigen and native T cells; the percentage of CD45RA-positive cells was low, and this result must be confirmed in a further examination.

Our present result indicated that high-dose methylprednisolone was effective in half of our patients with refractory anemia. High-dose methylprednisolone is valuable as a primary treatment before other immunosuppressive treatment because of its ease of use. High efficacy with high-dose methylprednisolone is highly expected, especially in patients in whom an increment in CD68-positive cells in bone marrow cells is observed.

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