met the following criteria: aged 18 to 75 years, newly diagnosed patients, no specific prior treatment for the disease, and severe AA. The disease was considered severe if at least 2 of the following were fulfilled: a neutrophil count of less than 0.5×10^9/L, a platelet count of less than 20×10^9/L, and a reticulocyte count of less than 20×10^9/L with hypocellular bone marrow.[16] Patients were excluded if they had been diagnosed as having Fanconi anemia or dyskeratosis congenita, severe uncontrolled infection, or malignancies. Cytogenetic studies were performed for all patients. We estimated a 30% difference in response rate between the G-CSF− group (ATG + CyA) and the G-CSF+ group (ATG + CyA + G-CSF). To detect a 30% difference, 45 patients per treatment group were required. Compensating for an estimated nonevaluability rate of 10%, it was considered reasonable to enroll at least 100 patients.

Informed written consent was obtained from all patients prior to study entry with Institutional Review Board approval at each of the participating centers and in accordance with the Declaration of Helsinki.

Treatment protocol

Patients were randomized to receive either ATG and CyA or ATG, CyA, and G-CSF. Horse ATG (Lymphoglobuline; Merieux, Lyon, France) was administered at a dose of 15 mg/kg per day for 5 days as a slow intravenous infusion over 12 hours. For the prevention of serum sickness, prednisolone was given orally at a dose of 1 mg/kg per day from day 1 to day 9, 0.5 mg/kg per day from day 10 to day 15, and 0.2 mg/kg per day from day 16 to day 21. CyA, given orally at a dose of 6 mg/kg per day, was started on day 1 and continued for at least 12 weeks. The dose was adjusted to achieve a whole-blood trough level of 150 to 250 ng/mL. In responders, CyA was continued for at least 28 weeks. In patients with a stable hematologic status for at least 4 weeks, gradual tapering of CyA (1mg/kg every 2 weeks) was permitted if hematologic data remained stable during the course of tapering. In patients randomized to receive G-CSF, filgrastim (Gran; Kirin-Sankyo, Tokyo, Japan) or lenograstim (Neutrogin; Chugai, Tokyo, Japan) was given intravenously at a dose of 400 μg/m^2 per day and 50 μg/kg per day, respectively, every other day until day 28, and then once or twice a week until day 84. The daily doses of filgrastim and lenograstim were those proved to be effective in clinical studies performed in Japan and approved by the Japanese Ministry for Health, Labor, and Welfare.[17,18] The primary end point of the study was the hematologic response at 12 weeks. 3 months, and 1 year after IST, and the secondary end points included the incidence of infections and febrile episodes during the first 12 weeks, survival rate, relapse rate, and incidence of the development of MDS /AL.

Evaluation of response and toxicity

Complete response (CR) was defined as a neutrophil count greater than 1.5×10^9/L, a platelet count greater than 150×10^9/L, and a hemoglobin level of greater than 110 g/L (11.0 g/dL). Partial response (PR) was defined by transfusion independence and no longer meeting criteria for severe disease.[19] Relapse was indicated by the requirement for blood transfusion. Toxicity of treatment was evaluated for the first 12 weeks and was graded according to the criteria of the World Health Organization.[20]

The Fisher Exact test was used to compare categoric variables, and the Mann-Whitney *U* test or the Student *t* test was used to compare continuous variables. The probability of survival and relapse was analyzed using the Kaplan-Meier method.[21] All statistical analyses were performed using SPSS 15.0 software (SPSS Japan, Tokyo, Japan).

Results

Patient characteristics

A total of 50 patients were randomized to receive ATG and CyA (G-CSF− group), and 51 patients were randomized to receive ATG, CyA, and G-CSF (G-CSF+ group). A total of 6 patients were excluded from analysis because of a diagnosis of lymphoma after randomization (1 patient), or treatment without ATG (5 patients) according to the patient's wishes after enrollment. Patient characteristics of the G-CSF+ and G-CSF− groups are summarized in Table 1. All patients, except 3 with hepatitis-associated AA, had no identifiable cause of AA (idiopathic AA). There were no significant differences between 2 groups in age, sex, hemoglobin level, neutrophil count, platelet count, reticulocyte count, number of patients with a neutrophil count of less than 0.2×10^9/L (ie, very severe AA [vSAA]), and interval between diagnosis and treatment. A total of 8 and 11 patients had a neutrophil count of more than 0.5×10^9/L in the G-CSF− group and in the G-CSF+ group, respectively.

Response

At 12 weeks, CR was observed in 2 (4%) patients, and PR was observed in 22 (47%) patients in the G-CSF− group, for an overall response rate of 51% (24 of 47 patients). In the G-CSF+ group, no patients had a CR and 28 (58%) patients had a PR for an overall response rate of 58% (28 of 48) (Table 2). There were no statistically significant differences in overall response rates at 12 weeks between the 2 groups ($P = .31$). At 6 months, the overall response rate increased from 51% to 57% in the G-CSF− group, and from 58% to 77% in the G-CSF+ group. The difference in overall response rates at 6 months between the 2 groups was statistically significant ($P = .03$). At 1 year, the overall response rate increased from 57% to 76% in the G-CSF− group, but did not change (from 77% to 79%) in the G-CSF+ group. There was no statistically significant difference in overall response rate at 1 year between the 2 groups ($P = .46$). In the G-CSF+ group, there were

Table 1. Patient characteristics

Characteristic	ATG + CyA	ATG + CyA + G-CSF	*P*
No. of patients randomized	50	51	—
No. of patients evaluable	47	48	—
Age, median y (range)	54 (19-75)	53 (19-74)	.55
Sex, male/female	21/26	23/25	.75
Cause of AA, no. patients			
Idiopathic	46	46	.51
Hepatisis	1	2	—
Hemoglobin, median g/L (range)	60 (35-82)	60 (31-84)	.67
Neutrophil count, median × 10^9/L (range)	0.32 (0.02-1.01)	0.30 (0.01-1.21)	.45
Platelet count, median × 10^9/L (range)	9 (1-38)	9 (1-31)	.62
Reticulocyte count, median × 10^9/L (range)	11 (0-65)	9 (0-35)	.08
No. of patients with a neutrophil count less than 0.2×10^9/L	11	19	.07
Interval between diagnosis and treatment, median d (range)	20 (3-152)	18 (1-112)	.89

— indicates not applicable.

Table 2. Response to treatment at 12 weeks, 3 months, and 1 year after treatment

Time after treatment	ATG + CyA, no. (%)	ATG + CyA + G-CSF, no. (%)	P
12 weeks			
No. of patients evaluable	47	48	—
CR	2	0	—
PR	22	28	—
Total response, CR+PR	24 (51)	28 (58)	.31
Death	0	2	—
6 months			
No. of patients evaluable	46	47	—
CR	3	2	—
PR	23	34	—
Total response, CR+PR	26 (57)	36 (77)	.03
Death	0	2	—
1 year			
No. of patients evaluable	41	47	—
CR	1	3	—
PR	30	34	—
Total response, CR+PR	31 (76)	37 (79)	.46
Death	1	2	—

— indicates not applicable.

no differences in overall response rate between the filgrastim-treated group and lenograstim-treated group (data not shown).

When the overall response rate was analyzed focusing on the vSAA patients (ie, a neutrophil count of less than 0.2×10^9/L), it was 27% (3 of 11 patients) at 12 weeks, 20% (2 of 10 patients) at 6 months, and 63% (5 of 8 patients) at 1 year in the G-CSF− group, and was 42% (8 of 19 patients) at 12 weeks, 63% (12 of 19 patients) at 6 months, and 63% (12 of 19 patients) at 1 year in the G-CSF+ group. Similar to the result obtained in total patients, overall response rate in patients with vSAA at 6 months but not at 12 weeks and 1 year was significantly higher in the G-CSF+ group compared with the G-CSF− group (12 weeks, $P = .34$; 6 months, $P = .03$; 1 year; $P = .68$).

The overall response rate for patients with a neutrophil count of more than 0.5×10^9/L at 12 weeks, 6 months, and 1 year was 50% (4 of 8 patients), 63% (5 of 8 patients), and 86% (6 of 7 patients) in the G-CSF− group, and 64% (7 of 11 patients), 64% (7 of 11 patients), and 91% (10 of 11 patients) in the G-CSF+ group, respectively. There was no significant difference in overall response between the 2 groups (12 weeks, $P = .45$; 6 months, $P = .51$; 1 year, $P = .64$). A total of 7 patients had chromosomal abnormalities at diagnosis, and 4 patients responded to IST.

When patients who responded to immunotherapy but needed continuous administration of CyA to maintain hematologic response were defined as CyA dependent, 8 patients in the G-CSF− group and 6 in the G-CSF+ group were included.

The median neutrophil counts at 4 weeks, 12 weeks, 6 months, and 1 year was 0.36×10^9/L, 1.32×10^9/L, 1.20×10^9/L, and 1.35×10^9/L in the G-CSF− group and 1.03×10^9/L, 1.19×10^9/L, 1.61×10^9/L, and 1.57×10^9/L in the G-CSF+ group, respectively. At 4 weeks but not 12 weeks, 6 months, and 1 year, the median neutrophil count in the G-CSF+ group was significantly higher compared with the G-CSF− group ($P = .004$).

A total of 3 patients (1 in the G-CSF− group and 2 in the G-CSF+ group) who failed to respond to initial therapy received a second course of IST; however, no patients responded. A total of 2 patients (both patients were in the G-CSF− group) who failed to respond to initial therapy received a BMT. One patient who received a transplant from an HLA-matched sibling is alive at 44 months after transplantation, but another patient who received a transplant from an HLA-matched unrelated donor died of pulmonary bleeding 2 months after transplantation.

Infectious complications

During the first 12 weeks, infections developed in 19 (40%) patients in the G-CSF− group and in 28 (58%) patients in the G-CSF+ group (Table 3). There was no significant difference in the proportion of patients with documented infection between the 2 groups ($P = .07$). Severe infections (grade 3 or 4) developed in 5 patients in the G-CSF− group and 8 patients in the G-CSF+ group, and the difference in the proportion of patients who contracted severe infections between the 2 groups was not statistically significant ($P = .29$). There were 31 infectious events in the G-CSF− group, including 6 severe infectious events such as bacteremia (5 events) and pneumonia (1 event), and 39 infectious events in the G-CSF+ group, including 11 severe infections such as bacteremia (7 events), pneumonia (3 events), and fungemia (1 event). There was no difference in the incidence of infectious events between the 2 groups ($P = .07$). Among 13 infectious events, including bacteremia, pneumonia, and fungal infection, observed in the G-CSF+ group, 7 (54%) occurred during the period of a neutrophil count less than 0.5×10^9/L.

To investigate the correlation between infection and the degree of neutropenia, the morbidity of infection in patients with vSAA and non-vSAA was compared, and was higher in patients with vSAA than in those with non-vSAA (61% in vSAA, 45% in non-vSAA); however, the difference was not statistically significant ($P = .07$).

The median number of febrile days (38°C or higher) was 4 days in both groups. Deaths due to infection during the first 12 weeks occurred in 2 patients (bacteremia and fungemia) in the G-CSF+ group.

Survival

The overall probability of survival at 4 years is 88% for the G-CSF− group and 94% for the G-CSF+ group, with a median follow-up period of 52 months (range, 1 to 78 months) and 54 months (range, 1 to 86 months), respectively (Figure 1). There was no significant difference in the overall probability of survival between the 2 groups ($P = .44$). In the G-CSF+ group, there were no differences in survival rate between the filgrastim-treated group and lenograstim-treated group (data not shown). There were 6 deaths in the G-CSF− group, and 4 in the G-CSF+ group. Causes of death were bacteremia (1 patient each in the G-CSF− and G-CSF+ groups), pneumonia (1 patient in the G-CSF− group), fungal infection (1 in the G-CSF+ group), intracranial hemorrhage (1 patient each in the G-CSF− and G-CSF+ groups), BMT-related toxicity (1 patient each in the G-CSF− and G-CSF+ groups), renal failure (1 in the G-CSF− group) and metastatic brain tumor (1 in the G-CSF− group).

Cytogenetic analysis and clonal disease

Before therapy, a clonal cytogenetic abnormality was detected in 7 (8%) of 91 evaluable patients (4 patients with −Y, 2 patients with deletion 13, and 1 patient with trisomy 8). Among 7 patients with cytogenetic abnormalities, 1 patient (−Y) randomized to receive G-CSF developed refractory anemia with ringed sideroblasts (RARS) at 14 months after treatment, and 2 patients (1 with −Y, 1 with trisomy 8) not randomized to receive G-CSF experienced the disappearance of clonal abnormalities during the follow-up period.

Table 3. Documented infections and febrile episodes during 12 weeks after initiation of treatment

	G-CSF⁻ group	G-CSF⁺ group	*P*
n	47	48	—
No. of patients with documented infections*; severe infection, grade 3 or 4	19; 5	28; 8	.07; .30
Accumulated number of infectious events†	31	39	.07
Bacteremia	5	7	—
Pneumonia	1	3	—
Upper respiratory tract infection	5	6	—
Intestinal infection	2	4	—
Urinary tract infection	1	0	—
Genital infection	0	2	—
Cellulitis	1	2	—
Herpes zoster	1	0	—
Herpes simplex	2	4	—
Fungal infection	0	3	—
Gingivitis	1	0	—
Fever of unknown origin	12	8	—
Febrile days over 38°C, median no. (range)	4 (1-17)	4 (1-38)	.83
Death due to infection, no. patients	0	2	.25

— indicates not applicable.
*Patients who had fever of unknown origin were included.
†Events of fever of unknown origin were included

In the G-CSF− group, chromosomal abnormalities appeared after treatment in 2 (6%) of 33 evaluable patients (1 with −Y and 1 with trisomy 8), with a subsequent disappearance of the chromosomal abnormality in 1 patient with −Y. In the G-CSF+ group, chromosomal abnormalities appeared after treatment in 6 (15%) of 41 evaluable patients (1 with −Y, 2 with monosomy 7, 1 with inv 7, 1 with trisomy 8, and 1 with monosomy 19), with a subsequent disappearance of the chromosomal abnormalities in 3 patients (1 with −Y, 1 with inv 7, and 1 with monosomy 19). All patients who developed chromosomal abnormalities after IST had revealed normal cytogenetics before IST.

There was no significant difference in the incidence of development of chromosomal abnormalities between the 2 groups ($P = .21$). In 8 patients in whom chromosomal abnormalities appeared after treatment, 2 patients (1 with −Y in the G-CSF− group and 1 with monosomy 7 in the G-CSF+ group) developed refractory anemia (RA).

As for the development of the clonal diseases, including MDS, AML, and paroxysmal nocturnal hemoglobulinemia (PNH), 1 patient developed RA at 38 months and 1 patient developed PNH at 25 months after treatment in the G-CSF− group, 2 patients developed MDS (RA and RARS) at 14 and 40 months, and 1 patient developed PNH at 41 months after treatment in the G-CSF+ group. No patients had definitive PNH before therapy. The presence of PNH was defined by a positive Ham test or loss of expression of CD55 and CD59 on red blood cells by flow cytometry. The overall risk for MDS/AML at 4 years is 3% for the G-CSF− group and 5% for the G-CSF+ group. There were no significant differences in the overall risk for MDS/AML between the 2 groups ($P = .63$). One patient who developed RA received peripheral blood stem-cell transplantation from an HLA-matched sibling and died of chronic graft-versus-host disease 5 months after transplantation.

Relapse

A total of 21 patients (15 in the G-CSF− group and 6 in the G-CSF+ group) relapsed after IST. In relapsed patients, all patients had received CyA at the time of relapse or had a history of CyA administration for at least for 6 months. The risk for relapse at 4 years was 42% in the G-CSF− group, and 15% in the G-CSF+ group (Figure 2). There was a significant difference in relapse rate between the 2 groups ($P = .01$). A total of 6 patients who relapsed after initial responses received a second course of IST, of whom 4 were in the G-CSF− group and 2 in the G-CSF+ group. Of the 6 patients 2 (33%) responded to the second therapy. Both responders belonged to the G-CSF+ group. A total of 2 patients (both in the G-CSF+ group) who relapsed after initial responses received BMT (1 from a HLA-matched sibling and another from an HLA-matched unrelated donor) and were alive at 41 months and 32 months after transplantation.

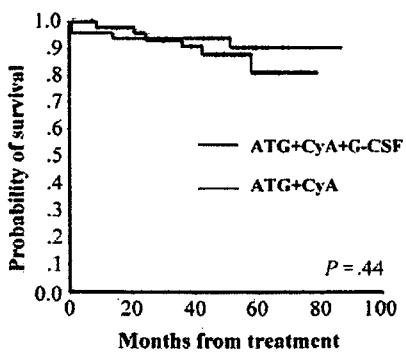

Figure 1. Actuarial survival of adult patients with SAA in the G-CSF− and G-CSF+ groups.

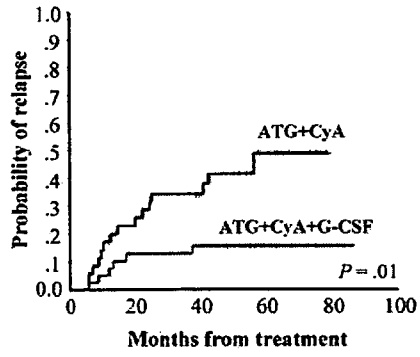

Figure 2. Cumulative incidence of relapse in adult patients with SAA in the G-CSF− and G-CSF+ groups.

Toxicity

The incidence of toxicity was comparable between G-CSF− and G-CSF+ groups. Acute allergic reaction during ATG therapy was observed in 81% of patients in the G-CSF− group and 92% of patients in G-CSF+ group ($P = .11$). A total of 2 patients in the G-CSF− group and 4 patients in the G-CSF+ group had serum sickness. Toxicity greater than grade III was noted in 2 patients (anaphylactic reaction to ATG and delirium associated with CyA administration) in the G-CSF− group, and in 1 patient (liver dysfunction related to ATG administration) in the G-CSF+ group.

Discussion

The results of this prospective multicenter study showed that the addition of G-CSF to IST plays some role in the treatment of SAA. The hematologic response rate at 6 months in the G-CSF+ group was significantly higher compared with the G-CSF− group (77% in the G-CSF+ group, 57% in the G-CSF− group), but at 1 year was comparable (79% in the G-CSF+ group, 76% in the G-CSF− group). This indicates that G-CSF can accelerate the recovery of hematopoiesis in patients with AA when used in combination with IST. Our result is inconsistent with a similar study conducted in Japanese children with SAA, which showed no difference between the G-CSF+ and the G-CSF− group in terms of hematologic response.[14] A European group also reported the result of a similar study in patients with SAA (age, 1-82 years), which showed that G-CSF only enhanced the recovery of neutrophils.[15] The reasons why different results were obtained in these studies are uncertain. However, it is possible that patient's age might influence the results, because the distribution of patient's age is apparently different among the studies.

Our study showed that the accumulated relapse rate was significantly lower in the G-CSF + group (42% in the G-CSF+ group and 15% in the G-CSF− group at 4 years; $P = .01$). This indicates that G-CSF can reduce the relapse rate in patients who have responded to IST. The reason why G-CSF has an impact on the occurrence of relapse is uncertain. However, as G-CSF has a stimulatory effect on the growth of hematopoietic stem and progenitor cells in AA,[22] it is possible that hematopoietic stem and progenitor cells, which can escape from the immune attack, might expand in patients who are successfully treated with IST in combination with G-CSF. If so, it is possible that relapse rate is low in the G-CSF+ group because of the high expansion of immune attack-resistant hematopoietic cells. A Japan childhood AA study group showed that there was a trend of low relapse rate in the G-CSF+ group (the risk for relapse at 4 years was 29% in the G-CSF+ group and 64% in the G-CSF− group), although the difference between the 2 groups was not statistically significant ($P = .10$), which might be due to a low number of patients enrolled.[14] A European group reported that the actuarial risk for relapse after IST was 35% at 10 years, and the relapse occurred at any time from a few months to 10 years after IST without a particular period of higher occurrence.[23] Because the median follow-up period of our study was not so long, the possibility that G-CSF only delays the time of relapse cannot be excluded. To elucidate whether G-CSF actually prevents relapse or only delays the time of relapse, further follow-up is required

To date, there was no significant difference between the G-CSF+ and the G-CSF− group in terms of overall survival (94% to 88% at 4 years). This finding is in keeping with that of previous reports.[14,15] It has been reported that the overall survival in patients who do not relapse is better than that of patients who relapse.[23] Therefore, better survival in the G-CSF+ group will be expected because of the low incidence of relapse in the G-CSF+ group. Further follow-up is necessary to conclude whether a difference in overall survival exists between the G-CSF+ and the G-CSF− group.

There was no difference in the incidence of documented infections and febrile episodes during the first 12 weeks between the G-CSF+ and G-CSF− groups, although the addition of G-CSF to IST resulted in an increase in the neutrophil counts. This result is in agreement with those of previous studies[14,15] suggesting that G-CSF has no preventive effect on infections during the IST. In our study, the proportion of patients who contracted infection was relatively higher in the G-CSF+ group compared with the G-CSF− group, although the difference between the 2 groups was not statistically significant (58% vs 40%; $P = .07$). This might be due to the relatively high proportion of patients with vSAA (neutrophil count, $< 0.200 \times 10^9$/L [200/μL]) in the G-CSF+ group compared with the G-CSF− group (40% in the G-CSF+ group and 23% in the G-CSF− group) who were more susceptible to infections.

Over the long term, patients with AA who have been treated with IST have an increased risk (10%-47%) of developing MDS or AML.[24] However, it is uncertain whether evolution to MDS/AML is a reflection of the natural history of AA or secondary disease related to IST. In addition, it has been reported that administration of G-CSF was associated with the development of MDS/AML,[25-27] although conflicting results have been reported.[28] In a retrospective study from Japan, it was demonstrated that 4 (22%) of 18 adult patients with AA patients treated with both IST and G-CSF developed MDS, and a high cumulative dose of G-CSF and the use of G-CSF for more than 1 year were the significant risk factors for developing MDS.[25] Another study showed that the cumulative incidence of developing MDS/AML was 13.7% in children with AA who received IST and danazol with or without G-CSF, and long-term use of G-CSF and no response to therapy at 6 months were significant risk factors for developing MDS.[26] In contrast, the Italian group showed that the risk for developing secondary malignancies at 60 months in patients treated with IST with or without G-CSF treatment was 9% and 7%, respectively ($P = .99$).[28] This study concluded that large doses of G-CSF (36 000 μg/patient) administered over a long period of time (6 months) in conjunction with IST do not increase the actuarial risk of developing MDS/AML. In our study, the risk for developing MDS/AML at 4 years is 3% for the G-CSF− group and 5% for the G-CSF+ group ($P = .63$). Our finding suggests that use of G-CSF with IST in a relatively short period of time (12 weeks) does not increase the risk for developing MDS/AML, at least during the several years after IST. Longer follow-up (at least 10 years) is necessary to determine the role of G-CSF in the development of MDS/AML. Until then, routine use of G-CSF is not recommended unless part of a clinical trial.

It is well known that chromosomal abnormalities develop in some patients after IST.[29] In addition, Japanese studies suggest a close relationship between the use of G-CSF and development of monosomy 7.[25,26] In our study, 8 (11%) of 74 evaluable patients who received IST developed chromosomal abnormalities, and there was no significant difference in the incidence of development of chromosomal abnormalities between the G-CSF+ and G-CSF− groups. This result is consistent with data from an Italian group.[28] In our study, however, it should be noted that monosomy 7 was only developed in the G-CSF+ group (2 patients), and 1 patient

with monosomy 7 developed MDS. It has recently been shown that G-CSF preferentially stimulates the proliferation of monosomy 7 cells expressing the class IV G-CSF receptor, which is defective in signaling cell maturation.[29]

A transient appearance of chromosomal abnormalities in patients with AA after IST is also a well-documented phenomenon.[28,30,31] An Italian group showed 9 patients (4 in the G-CSF+ group, 5 in the G-CSF− group) with transient chromosomal abnormalities.[28] In the present study, transient chromosomal abnormalities were observed in 4 patients (3 in the G-CSF+ group, 1 in the G-CSF− group). Therefore, the appearance of abnormal cytogenetic clones after IST does not necessarily mean the subsequent expansion of those clones, and appeared to be unrelated to the combined use of G-CSF.

The present study suggests that combined use of immunosuppressive agents and G-CSF has some benefits in terms of the promotion of hematopoietic recovery and suppression of relapse rate, which result in reducing the need for subsequent treatments such as blood transfusion and second IST. G-CSF support of IST might be feasible for the treatment of adult SAA; however, further follow-up is required to elucidate whether G-CSF increases the risk for MDS/AML. In addition, it is important to discuss whether G-CSF support of IST is appropriate in terms of cost-effectiveness. To address this issue, long-term follow-up is necessary.

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Authorship

Author contributions: M.T., S.N., A.U., M.O., and H.M. designed research; M.T analyzed data and drafted the paper; A.K., S.I, and Y.Y. contributed to the enrollment of patients.

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Cyclosporine Therapy for Acquired Aplastic Anemia: Predictive Factors for the Response and Long-term Prognosis

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Abstract

Although cyclosporine (CsA) is a key drug in the treatment of acquired aplastic anemia (AA), the role of single-agent therapy with CsA remains unclear. To determine the efficacy of CsA in the treatment of AA, we treated 38 AA patients with CsA alone and followed up the patients for 6 months to 16 years. Twenty patients (53%) achieved either a partial or complete remission within 1 year of starting CsA therapy. Thirteen (81%) of 16 patients who showed an increase in the reticulocyte count of $>20 \times 10^9$/L within 2 months achieved remission, whereas the response rate was only 32% in patients who failed to show such an increase in the reticulocyte count. The actuarial overall survival and failure-free survival rates at 5 years were 91% and 37%, respectively. These data indicate that CsA alone can achieve a sustained remission in approximately 40% of AA patients, with a low probability of inducing secondary clonal diseases. Given its low toxicity and because the effectiveness of CsA can be judged within 2 months of therapy, CsA may be the first drug of choice at outpatient clinics for AA patients not requiring transfusions.

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Key words: Aplastic anemia; Cyclosporine; Monotherapy; Reticulocytes; Predictive factor

1. Introduction

Cyclosporine (CsA) has been successfully used for the treatment of acquired aplastic anemia (AA) as a single agent [1-3] or in combination with antithymocyte globulin (ATG) [4-6]. A randomized controlled study conducted by a French group clarified that CsA as single-agent therapy and CsA therapy with ATG are equally effective in restoring hematopoietic function in patients with severe AA [3]. CsA has not been used alone for the treatment of severe AA, however, because a subsequent controlled study demonstrated the superiority of combination therapy with ATG plus CsA compared with ATG alone in the treatment of severe AA [4]. Another controlled study by the European Group for Blood and Marrow Transplantation (EBMT) showed that therapy with ATG plus CsA led to a higher rate of remission than with CsA alone in patients with nonsevere AA [7]. CsA is therefore considered a supplemental drug rather than a main drug for the treatment of AA, and the role of CsA as single-agent therapy has not attracted much attention.

Monotherapy with CsA has several advantages over therapy with ATG and androgen. Because its toxicity is lower than with ATG, CsA can be administered safely at outpatient clinics, and its use does not necessitate platelet transfusions, which are often necessary when ATG is administered. CsA does not cause virilism as androgen does. Therefore, CsA may be the treatment of choice for AA patients who do not require transfusions but do show progressive pancytopenia. We have treated AA patients with CsA alone since the late 1980s because of the unavailability of ATG in Japan until 1995; thereafter, we used CsA only in cases not requiring transfusions. To determine the role of CsA monotherapy in the treatment of AA, we analyzed its effectiveness and the long-term prognosis of patients treated with this therapy.

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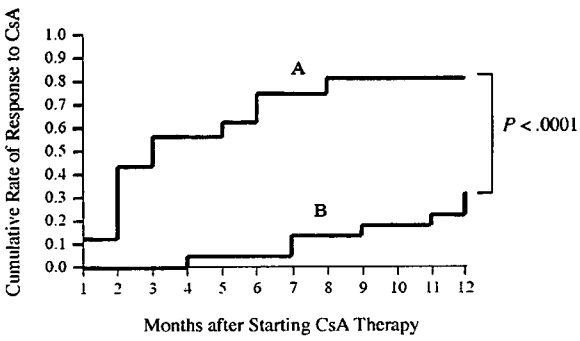

Figure 1. Cumulative rate of response to cyclosporine (CsA). Patients were divided into 2 groups according to the reticulocyte response, which was defined as an increase of $\geq 20 \times 10^9$/L in the reticulocyte count from the pretreatment value, and cumulative rates of response to CsA for responders (A) and nonresponders (B) were compared with respect to reticulocyte numbers.

2. Patients and Methods

2.1. Patients

We treated 38 patients with acquired idiopathic AA (9 with severe disease, 29 with moderate disease) ranging in age from 16 to 82 years (median, 53 years) from October 1986 to December 2005 [8]. The severity of AA was graded according to the criteria proposed by Camitta et al [9]. The male-female ratio was 21:17. All patients had a neutrophil count $>0.2 \times 10^9$/L. The time from AA diagnosis to CsA therapy ranged from 0 to 271 months (median, 6 months). Fourteen patients (37%) were dependent on red blood cell transfusions at the initiation of therapy. The reasons for not receiving ATG as first-line therapy were the unavailability of ATG in Japan at the time of diagnosis in 18 patients and the absence of a transfusion requirement in 17 patients.

2.2. Treatment with CsA

All patients received 3 to 5 mg/kg of CsA for at least 3 months. Patients who did not show any signs of improvement at the completion of 3 months of therapy were changed to either ATG or androgen therapy in combination with CsA therapy. The response to therapy was assessed by the criteria proposed by Camitta [10]. A complete response (CR) was defined as a hemoglobin concentration normal for the patient's age, a neutrophil count $>1.5 \times 10^9$/L, and a platelet count $>150 \times 10^9$/L. A partial response (PR) was defined for patients with severe AA as being transfusion independent and no longer meeting the criteria for severe disease. In patients with nonsevere AA, a PR was defined as transfusion independence (if the patient previously had been transfusion dependent), the doubling or normalization of at least 1 cell line, or an increase in the baseline hemoglobin level by >3 g/dL (if initially <6 g/dL), in the neutrophil count to $>0.5 \times 10^9$/L (if initially $<0.5 \times 10^9$/L), and in the platelet count to $>20 \times 10^9$/L (if initially $<20 \times 10^9$/L).

2.3. Detection of Paroxysmal Nocturnal Hemoglobinuria–Type Cells

To detect paroxysmal nocturnal hemoglobinuria (PNH)-type granulocytes, we used phycoerythrin-labeled anti-CD11b monoclonal antibodies (MoAbs) (BD Medical Systems, Mountain View, CA, USA), fluorescein isothiocyanate (FITC)-labeled anti-CD55 (clone IA10, mouse immunoglobulin G2a [IgG2a]; BD Pharmingen, San Diego, CA, USA), and FITC-labeled anti-CD59 (clone p282, mouse IgG2a; BD Pharmingen) in combination with isotype-matched control antibodies, as described in our previous report [11]. For the analysis of PNH-type red blood cells, we used phycoerythrin-labeled antiglycophorin A MoAb (clone JC159; Dako, Glostrup, Denmark) instead of using antiglycophorin A MoAb, FITC-labeled anti-CD55, and anti-CD59 MoAb on ice for 25 minutes. We analyzed at least 105 $CD11B^+$ granulocytes and glycophorin A–positive red blood cells within each corresponding gate by means of FACScan flow cytometry (BD Medical Systems).

2.4. Determination of DRB1 Alleles

The DRB1 alleles of the 38 AA patients were determined by means of polymerase chain reaction analysis with sequence-specific primers (Micro SSP HLA DNA typing trays; One Lambda, Canoga Park, CA, USA). Genomic DNA was prepared from blood samples with a DNA-extraction kit (Generation Capture Column Kit; Gentra Systems, Minneapolis, MN, USA).

2.5. Statistical Analysis

Logistic regression was used in both univariate and multivariate analyses to assess factors that predict the response to CsA. The following clinical parameters were analyzed to determine their relationship with the response to CsA: age, time to CsA therapy from diagnosis, the presence of HLA-DRB1*1501, the presence of PNH-type cells, an increase in the reticulocyte count, and disease severity. The Kaplan-Meier method graphically compared the cumulative incidences of the response to CsA therapy and the times to event, and any differences in reticulocytes between responders and nonresponders were assessed by the log-rank test. All statistical analyses were performed with the JMP software package, version 5.0.1J (SAS Institute, Cary, NC, USA).

3. Results

3.1. Response Rate

The patients were followed up from 6 months to 16 years (median, 6.5 years) after CsA therapy. The rate of CR plus PR responses to CsA was 34% at 6 months and 53% at 12 months. Only 1 patient achieved a CR by the completion of 12 months of treatment. The response rates in severe AA and nonsevere AA patients were 44% and 55%, respectively. The reticulocyte count increased by $>20 \times 10^9$/L from the pretreatment value in 42% of the patients, and the increase in reticulocytes occurred within 2 months of CsA

Table 1.
Relationship between Clinical Parameters and Response to Cyclosporine (CsA)*

	Relative Risk (95% CI)	P
Age	0.036 (0.0001-4.67)	.18
Time from diagnosis to CsA therapy	1.43 (0.108-20.5)	.77
HLA-DRB1*1501	1.84 (0.223-17.6)	.56
PNH-type cells	6.24 (0.875-77.88)	.09
Increase of reticulocytes	9.07 (1.327-113.3)	.04
Dependency on transfusion	1.00 (0.09-9.03)	.99

*In this multivariate analysis, AA severity was classified according to the dependency on transfusions. CI indicates confidence interval; PNH, paroxysmal nocturnal hemoglobinuria.

therapy in these responders. The cumulative rate of achieving a PR or CR in the reticulocyte-count responders by 1 year of therapy was 81%, which was significantly higher than the 32% rate for nonresponders regarding the reticulocyte count (Figure 1).

The administration of CsA was continued for more than 2 years in 14 (70%) of 20 responders. Eleven patients (55%) achieved an unmaintained remission after ceasing CsA treatment, and 6 patients (30%) required a low CsA dose (40-150 mg/day) because of the dependency of their hematopoietic function on CsA. AA relapsed in 5 patients (25%) between 3 and 6.5 years after the cessation of CsA therapy. Two of the patients responded to a reinitiation of CsA therapy and thereafter developed CsA dependency.

3.2. Factors Affecting the Response to Therapy

Several parameters, such as the presence of PNH-type cells [12] and HLA-DRB1*1501 [13], have been suggested to demonstrate a good response to immunosuppressive therapy. Recent reports revealed a higher prevalence of increases in PNH-type cells in AA patients than had previous reports [12,14]. When we examined these parameters in our patients, 19 (50%) of 38 patients had small populations of PNH-type cells, whereas 11 (29%) of 38 patients possessed HLA-DRB1*1501. The response rates to CsA therapy were 68% in patients with a small population of PNH-type cells and 37% in patients without PNH-type cells (P = .051); the response rates were 55% with DRB1*1501 and 52% without DRB1*1501 (P = .88). Table 1 summarizes the relationship between the clinical parameters, including dependency on transfusions, and the response to CsA. A multivariate analysis revealed that only an increase in the reticulocyte count within 2 months of CsA therapy predicted a good response.

3.3. Prognosis

The disease of 1 patient (2.6%) developed into PNH after 5 years of CsA therapy, and none of the patients later developed myelodysplastic syndrome or acute myeloid leukemia. Gastric cancer and cholangiocarcinoma in 1 patient each, and these 2 patients died 13 years and 7 years, respectively, after starting CsA therapy. The actuarial overall survival and failure-free survival rates at 5 years were 91% and 37%, respectively (Figure 2). The time to treatment failure was defined as the time from the first day of treatment until salvage treatment for nonresponse, the occurrence of relapse, the development of a clonal hematologic disease or solid tumor, or disease-related or treatment-related death, whichever came first.

4. Discussion

This retrospective analysis revealed that treatment with CsA alone can achieve a remission in approximately 50% of AA patients, regardless of disease severity. The response rate is similar to that reported by Hinterberger-Fischer et al [1] but is slightly higher than the rates reported by Leonard et al and Gluckman et al [2,3]. The better response rate in our study is probably because all patients received CsA as the first therapy and no patient in this study had very severe AA, as defined by a neutrophil count of $<0.2 \times 10^9$/L.

Among several factors that may affect the response to CsA, only an increase in the reticulocyte count within 2 months of therapy was associated with a good response to CsA. Although we previously reported that the presence of HLA-DRB1*1501 predicts a favorable response to CsA [13], the current study could not confirm this finding. This discrepancy may be due to the inclusion of patients who had a long history of AA. Illness duration is known to negatively affect the response to immunosuppressive therapy [15]. Although some patients carried HLA-DRB1*1501, the long disease duration before they started to receive CsA may have limited their responsiveness to CsA. We recently demonstrated that the presence of small populations of PNH-type cells is associated with a good response to therapy with ATG plus CsA in AA patients [12]; however, the present study failed to show such a role for the presence of PNH-type cells in predicting a good response to CsA. CsA alone may not be potent enough to eradicate the immune mechanisms and restore hematopoiesis in AA patients.

Clonal hematopoietic diseases such as PNH and myelodysplastic syndrome have been reported to eventually develop in 10% to 15% of AA patients treated with immunosuppressive

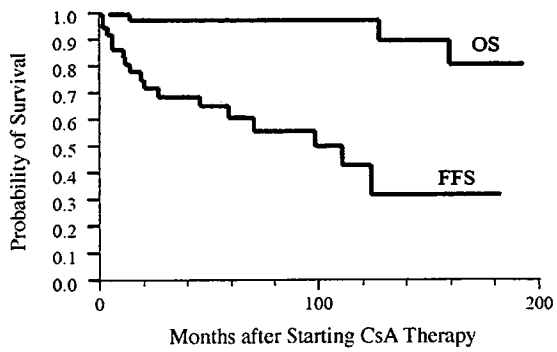

Figure 2. Prognosis after cyclosporine (CsA) therapy. OS indicates overall survival; FFS, failure-free survival.

therapy [16,17]. A study of pediatric AA patients by a Japanese group found that the risk of developing myelodysplastic syndrome in AA patients who received CsA and granulocyte colony-stimulating factor was significantly higher than in patients who did not receive these drugs [18]. In the present study, PNH developed in only 1 patient, and myelodysplastic syndrome or acute myeloid leukemia developed in none of the patients. Although the study population was relatively small and the follow-up period was short, our findings suggest that monotherapy with CsA may not be associated with an increased risk of developing clonal disorders in AA patients. On the other hand, solid tumors later developed in 2 patients who had received CsA for many years (13 years and 7 years). A recent follow-up study of AA patients who had undergone immunosuppressive therapy revealed a relatively high risk of developing solid tumors [17]. It therefore seems necessary to carefully observe AA patients on CsA therapy to identify any occurrence of malignancies.

Responders to CsA are believed to often require continuous drug administration to maintain remission [13]. This dependency has been thought to be one of the drawbacks of CsA therapy. A recent study by the EBMT group reported that only 38% of responders to therapy with ATG plus CsA no longer needed CsA at 5 years after therapy [19]. In our patients treated with CsA alone, however, the cumulative rate for patients no longer needing CsA at 5 years was 55%. Therefore, the rate of developing CsA dependency in responders to CsA therapy may not be as high as originally expected. The long-term administration of CsA without any apparent necessity thus should be avoided in order to prevent chronic nephrotoxicity and secondary malignancies that may occur as a result of CsA-induced immunodeficiency. The results of our study warrant further attempts to taper the CsA dosage in all responders to CsA.

The results of this analysis have important implications for the management of patients with moderate or severe AA who do not require transfusions. ATG plus CsA is the standard therapy for AA. All AA patients who need transfusions and are ineligible for allogeneic stem cell transplantation should undergo this immunosuppressive therapy as soon as possible. However, many patients with moderate AA and some with severe AA do not need transfusions at the time of AA diagnosis, and because of an absence of symptoms, these patients usually do not want to be hospitalized. CsA treatment on an outpatient basis is the therapy of choice for such patients, because a response rate of approximately 40% can be expected without causing any significant toxicity. A change of therapy should be considered unless the reticulocyte count increases within 2 months. The effectiveness of CsA for the treatment of AA patients not requiring transfusions still needs to be confirmed by prospective studies.

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Long-term outcome of patients with acquired primary idiopathic pure red cell aplasia receiving cyclosporine A. A nationwide cohort study in Japan for the PRCA Collaborative Study Group

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ABSTRACT

Background and Objectives

Cyclosporine A (CsA) has become one of the leading agents for the treatment of pure red cell aplasia (PRCA). However, further studies are necessary to determine the relapse-free survival (RFS) and overall survival (OS) of patients treated with this drug, the minimum duration of therapy for induction of remission, and whether or not there is need for maintenance treatment.

Design and Methods

We conducted a nationwide survey in Japan. From a total of 185 patients (with 73 primary idiopathic PRCA and 112 with secondary PRCA), we evaluated 62 patients with primary idiopathic PRCA for this report.

Results

The remission induction therapy for these patients included CsA (n=31), corticosteroids (CS) (n=20) or other drugs (n=11). CsA and CS produced remissions in 23 (74%) and 12 (60%) patients, respectively. The salvage treatment produced remissions in 58 patients (94%). Forty-one and 15 patients were maintained on CsA±CS (CsA-containing group) or CS alone (CS group), respectively. The median RFS in the CsA-containing group was 103 months, longer than that seen in the CS group (33 months) ($p<0.01$). Of 14 patients whose CsA was discontinued, 12 patients (86%) relapsed after a median of 3 months (range 1.5 to 40 months), while only 3 of 27 patients (11%) relapsed during CsA-containing maintenance therapy. Thus, the discontinuance of maintenance therapy was strongly correlated with relapse ($p<0.001$). Four patients in the CsA-containing group died; however, the OS of this group was not significantly different from that of the CS-groups ($p=0.104$).

Interpretation and Conclusions

CsA-containing regimens sustain prolonged RFS more effectively than CS in primary idiopathic PRCA and seem to be important to prevent relapse.

Key words: pure red cell aplasia, cyclosporine A, relapse-free survival, maintenance therapy.

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Pure red cell aplasia (PRCA) is characterized by severe normochromic, normocytic anemia associated with reticulocytopenia and absence of erythroblasts from an otherwise normal bone marrow.[1-4] The acquired form of chronic PRCA may present as a primary hematologic disorder in the absence of any other disease, or secondary to neoplasms, infections, collagen vascular diseases, chronic hemolytic anemias, or after exposure to a variety of drugs and chemicals. Primary or secondary PRCA not responding to treatment of the underlying diseases is treated as an immunologically-mediated disorder.[1-4] Remissions have been achieved by treatment with corticosteroids (CS), cyclophosphamide, cyclosporine A (CsA), anti-thymocyte globulin (ATG), splenectomy, and plasmapheresis.[1-7] More recently, the anti-CD20 monoclonal antibody rituximab[8,9] and the anti-CD52 monoclonal antibody alemtuzumab (campath-1H)[10] have been reported to induce the remission of therapy-resistant PRCA. In general, remission induction can be easily achieved in the majority of patients. However, in the era before CsA became available, Clark *et al.* clearly showed that 80% of patients relapsed during the 24 months after having achieved remission.[11] Up to the present, the efficacy of CS, cyclophosphamide and CsA for patients with primary or secondary PRCA has been reported to be between 30-56%, 7-20% and 75-87%, respectively.[1-7,11] CsA has become established as one of the leading agents for the treatment of PRCA since the first, successfully treated cases in 1984.[12] However, it is unclear how many patients treated with CsA achieve a sustained remission and how many relapse. Up to the present, very few studies on the long-term follow-up of patients treated with CsA have been reported. Moreover, comparing one therapeutic approach to another for the treatment of PRCA is almost impossible since this disease is so rare that controlled studies are practically impossible to perform. We, therefore, conducted a nationwide survey of PRCA cases in Japan to elucidate the current status of immunosuppressive therapy for PRCA.

Design and Methods

Patients

The first questionnaires were sent to hematology departments in Japan to estimate the number of patients, aged 15 years and over, with newly diagnosed acquired chronic PRCA, excluding those with human parvovirus B19 infection. Secondary questionnaires were sent to collect data on underlying diseases, laboratory findings (including peripheral blood cell count with reticulocyte count and leukocyte differentials), findings of bone marrow examinations, immunological and cytogenetic parameters and the efficacy and the side effects of immunosuppressive therapy. Secondary questionnaires did not collect information on the trough concentration of CsA. The recommended dose of CsA in Japan is 6 mg/kg, to provide a trough concentration ranging from 150 to 250 ng/mL. The first period of the survey was between January 1990 and December 2004 across 47 institutions and the second period was between January 1990 and March 2006 across 109 institutions, including a follow-up survey of the patients identified in the first period. All combined, a total of 273 patients were enrolled from 45 institutions in response to the first questionnaires. A total of 185 patients were enrolled in response to the second questionnaires.

Classification of PRCA

There are several proposed classifications of PRCA. One is based on pathophysiology[5] and another, on underlying diseases.[2] The prognosis of patients with PRCA, which is one of the most important end-points of this study, depends on the nature of their underlying diseases.[11] We, therefore, classified our patients with PRCA based on their underlying diseases, according to the classification proposed by Dessypris and Lipton[2] with some modifications. In this classification, primary PRCA comprises preleukemic, autoimmune and idiopathic forms. The patients with definite cytogenetic abnormalities were classified as having secondary PRCA as either myelodysplastic syndrome (MDS) or preleukemia. Primary autoimmune PRCA is defined as the cases in which an immune pathogenic mechanism can be established by *in vitro* assay. Secondary questionnaires did not collect information on *in vitro* assays, therefore, cases of idiopathic PRCA in this study may include primary autoimmune PRCA.

Data analysis

The secondary questionnaires collected data on the reticulocyte count and a bone marrow examination at onset of aplasia but not at recovery. Remission was defined as no need for any further transfusions, whereas relapse was defined as the need to receive transfusions. The period to achieve maximum response varied from patient to patient; therefore, the date of remission was defined as that of the last transfusion after the initiation of remission induction therapy. Complete remission (CR), partial remission (PR) and no response (NR) were defined as the achievement of normal hemoglobin levels without transfusion, the presence of anemia without transfusion dependence, and the continued need for transfusions, respectively. It is difficult to determine the efficacy of each agent precisely when the patients are either concomitantly or sequentially treated with several agents. Moreover, the first agent(s) given may contribute to the efficacy of the agent(s) given subsequently. Therefore, in this study, the efficacy of the agent(s) reported in secondary questionnaires was re-evaluated according to the following criteria. In a simultaneous combination, the efficacies of all of the agents were determined as the same. In sequential administration and in a later on combination, the efficacy of each agent was determined depending on the response obtained during the period of administration, except for ATG and methylprednisolone. ATG and methylprednisolone usual-

ly do not produce immediate remission; therefore, the efficacies of these agents were evaluated together with the agent(s) used concomitantly and/or sequentially. The minimum period required for an evaluation of the response of an agent was defined as 2 weeks; therefore, an agent combined later on, within 2 weeks, was, for the purposes of the analysis, considered a simultaneous combination with the preceding agent(s).

Regarding maintenance treatment, the patients were classified according to the agent used for maintenance therapy as receiving CsA±CS (CsA-containing group) or CS alone (CS group) regardless of the agent(s) used for successful remission induction. The agents for remission induction and salvage therapy were defined as those used initially and those used either sequentially or in a later on combination, respectively. The agent for maintenance therapy was defined as that used or tailed off after successful remission induction. The RFS was estimated as transfusion-free survival. The overall survival and RFS were estimated by the Kaplan-Meier method and statistical differences were calculated by the log-rank test and χ^2 test.

Results

Classification of PRCA

According to the criteria of Dessypris and Lipton,[2] of the total of 185 collected patients with PRCA, 73 (39%) were classified as having primary idiopathic PRCA and 112 (61%) as having secondary PRCA (Table 1). From the 73 patients with primary idiopathic PRCA, 11 patients were excluded from further analysis because of insufficient data (nine patients) or too short an observation period after initiation of immunosuppressive therapy (two patients; 1 and 8 days of observation). Finally, 62 patients with primary PRCA were eligible for further analysis. The patients' age at the onset of anemia ranged from 18 to 89 years (55±18, mean ± standard deviation, SD) with a 23:39 (1:1.7) male to female ratio (Figure 1). The year at onset of PRCA was 1998±5 (mean±SD), ranging from 1990 to 2005.

Rate of response to the remission induction therapy

The remission induction therapy for these patients included CsA (n=31), CS (n=20), cyclophosphamide (n=3), anabolic steroids (n=1), or a simultaneous combination of CsA and anabolic steroids or CS (n=7) (Figure 1 and Table 2). CsA, as a remission induction therapy, produced CR or PR in 23/31 patients (74%). The initial dose of CsA for the responding patients was 4.8±1.2 mg/kg (mean±SD, n=23) with a range of 2.9 to 7.6 mg/kg body weight, which was higher than that for non-responding patients (3.9±1.3 mg/kg with a range of 2.1 to 5.6 mg/kg, n=8), although the difference was not statistically significant. When the patients who were treated with CsA alone were evaluated (n=23), the time for transfusion-independence from the start of therapy was 82±200 days (range, 0 to 910 days). Fifteen patients (65%) achieved transfusion-independence within 2 weeks, 17 patients (74%) within 1 month, 18 patients (78%) within 3 months and 20 patients (87%) within 6 months. CS, as a remission induction therapy, produced a CR or PR in 12/20 patients (60%). The initial dose of prednisolone in patients who responded to CS was 0.8±0.2 mg/kg (mean±SD, n=12) with a range of 0.5 to 1.0 mg/kg. There was no significant difference in the dose between the responders and non-responders. When the patients who were treated by CS alone were evaluated (n=9), the time for transfusion-independence from the start of therapy was 65±101 days (range, 0 to 311 days). Three patients (33%) achieved transfusion-independence within

Table 1. Classification of 185 patients with aquired pure red cell aplasia.

Causes of pure red cell aplasia	*Patients* *Number*	*Percent*
Primary		
Idiopathic	73	39.5%
Secondary, associated with		
Thymoma	42	22.7%
Hematologic malignancies		
Chronic lymphocytic leukemia		
B-cell type	1	0.5%
Large granular lymphocyte leukemia	14	7.6%
Macroglobulinemia	3	1.6%
Malignant lymphoma	8	4.3%
Myelodysplastic syndrome	11	5.9%
Acute myeloblastic leukemia	1	0.5%
Preleukemic	1	0.5%
Solid tumors	5	2.7%
Autoimmune, collagen vascular diseases		
Rheumatoid arthritis	7	3.8%
Systemic lupus erythematosus	1	0.5%
Systemic sclerosis	1	0.5%
Sjögren's syndrome	2	1.1%
Polymyalgia rheumatica	1	0.5%
Autoimmune hemolytic anemia	1	0.5%
Evans' syndrome	1	0.5%
Type 1 diabetes mellitus	1	0.5%
Myasthenia gravis	1	0.5%
Chronic thyroiditis	1	0.5%
Autoimmune hepatitis	2	1.1%
Drugs	2	1.1%
Chronic renal failure	5	5.7%

Table 2. Response to remission induction therapy.

Initial agent(s)	*No. of patients*	*Response, No. (%)* *CR*	*PR*	*CR+PR*	*NR*
CsA	31	10 (32%)	13 (42%)	23 (74%)	8 (26%)
CS	20	4 (20%)	8 (40%)	12 (60%)	8 (40%)
CY	3	0	0	0	3 (100%)
AS	1	0	0	0	1 (100%)
CsA+CS	4	0	4 (100%)	4 (100%)	0
CsA+AS	1	0	1 (100%)	1 (100%)	0
CS+AS	2	1 (50%)	1 (50%)	2 (100%)	0
Total	62	15 (24%)	27 (44%)	42 (68%)	20 (32%)

CsA: cyclosporine A; CS: corticosteroid including methyl-prednisolone and prednisolone; CY: cyclophosphamide; AS: anabolic steroid; CR: complete remission; PR: partial remission; NR: no response.

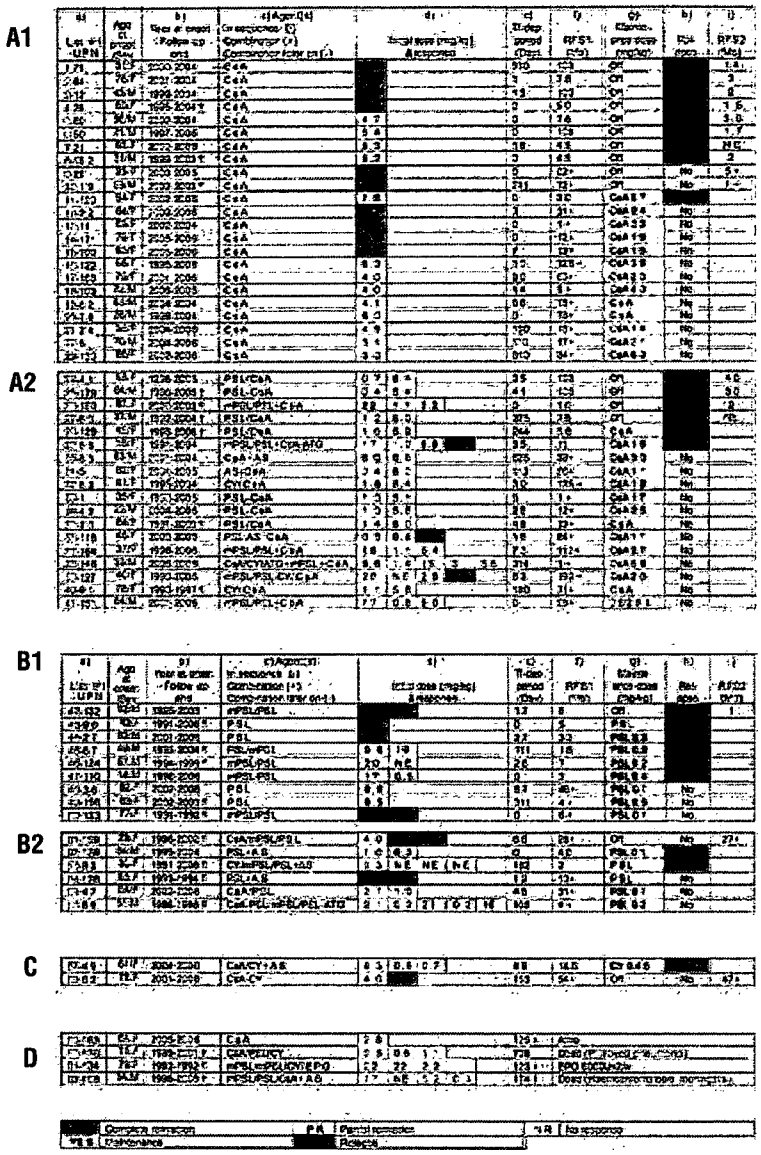

Figure 1. Immunosuppressive therapy in patients with primary idiopathic PRCA. (A) Cyclosporine A (CsA)-containing group: (A1) CsA alone, (A2) CsA in combination with other agents. (B) corticosteroid (CS) group: (B1) CS alone, (B2) CS in combination with other agents. (C) cyclophosphamide (CY) group. (D) Transfusion-dependent patients (non-responders). Abbreviations in each column; a) List #1; list number in Figure 1 and UPN (unspecified patient's number), b)¶Year at end of follow-up; †Death, c) agents are listed in order, (/); in sequential administration, (+); in simultaneous combination, (-); in combination later on, CsA; cyclosporine A, PSL; prednisolone, mPSL; methylprednisolone pulse therapy; ATG; antithymocyte globulin, CY; cyclophosphamide, AS; anabolic steroid, d) The initial dose and response to the agent; the order of agents corresponds to that shown in column c) and doses indicated are in mg/kg body weight/day, the color of each box shows response as indicated in the figure, e) Transfusion-dependent period (days) after the initiation of remission induction therapy, NE; not evaluable, f) RFS1; relapse-free survival (months) estimated as transfusion-free survival is shown as the period before the discontinuation of maintenance therapy, g) Off; tapered off, ‡doses of prednisolone/CsA in order, h) Relapse was defined as reappearance of transfusion requirement, i) RFS2; RFS after the discontinuation of maintenance therapy. EPO; erythropoietin.

2 weeks, six patients (67%) within 1 month and eight patients (89%) within 6 months. A simultaneous combination of CS and CsA produced remission in 4/4 patients. Cyclophosphamide was tried in three patients at a dose of 0.3 to 1.8 mg/kg, but no obvious responses were observed.

Salvage therapy

Twenty patients failed to respond to remission induction therapy. The effective salvage therapies for these patients are summarized in Figure 2 in which the period of administration of the initial agent(s) is also shown. The remission induction agent was rapidly discontinued in several patients. Among eight patients who failed to respond to initial CsA, six patients responded to CS (Figure 1-B2, 51-159, 55-47 and 56-69), cyclophosphamide (Figure 1C, 57-49 and 58-62), or a simultaneous combination of ATG+methylprednisolone+CsA (Figure 1-A2, 38-146). Of the remaining two CsA non-responders, one patient (Figure 1D, 59-163) was treated with CsA at a dose of 2.8 mg/kg/day but was still transfusion-dependent after 125 days, and the other did not respond to salvage therapies with a sequential administration of prednisolone and cyclophosphamide and eventually died due to *Pneumocystis jiroveci* pneumonia (Figure 1D, 60-138). Eight patients did not respond to CS; five patients responded to CsA (Figure 1-A2, 24-41, 25-179, 27-80, 28-129 and 35-20) and one other responded to cyclophosphamide (Figure 1-A2, 39-127). Of the remaining two CS non-responders, one patient was lost to the follow-up during the administration of erythropoietin (Figure 1D, 61-134), and the other did not respond to salvage therapies with a combination of CsA and anabolic steroids, received 240 units of red blood cell transfusion, and eventually died due to bacterial meningitis (Figure 1D, 62-158). There were three patients who did not respond to cyclophosphamide; two patients responded to CsA (Figure 1-A2, 32-63 and 40-81) and the other

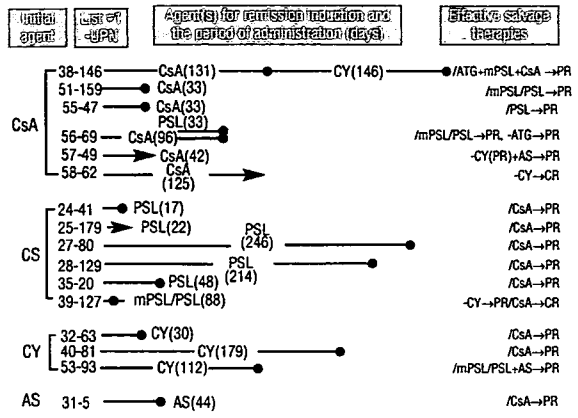

Figure 2. Effective salvage therapies for patients who failed to respond to the remission induction therapy. The initial agent(s) that failed to produce remission was discontinued (-o) or continued (→). Agents for salvage therapy were started in combination later on with the initial agent (-), simultaneously (+) or sequentially (/). The abbreviations are the same as those in the legend to Figure 1.

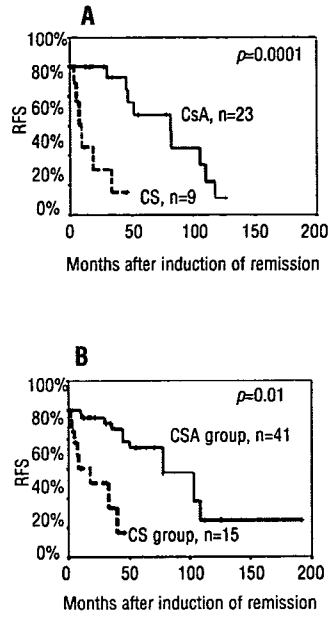

Figure 3. Relapse-free survival (RFS) of patients with primary idiopathic PRCA. RFS after induction of first remission was estimated as transfusion-free survival. A. The RFS of the patients treated with cyclosporine A (CsA) alone (solid line) (Patients listed in Figure 1-A1, n=23) is compared to that of the patients treated with corticosteroids (CS) alone (broken line) (Patients listed in Figure 1-B1, n=9). B. The RFS in the CsA-containing group (solid line) (patients listed in Figure 1-A1+A2, n=41) is compared to that of the CS group (broken line) (patients listed in Figure 1-B1+B2, n=15). There was a statistically significant difference between the duration of remission in the two groups based on the generalized Wilcoxon's test ($p<0.0001$ for A and $p<0.01$ for B).

responded to a combination of CS and anabolic steroids (Figure 1-B2, 53-93). A patient who was refractory to anabolic steroids responded to CsA (Figure 1-A2, 31-5). Finally, 58/62 patients (94%) with primary idiopathic PRCA responded to immunosuppressive therapy.

Relapse-free survival

Figure 3A illustrates the duration of RFS of the patients treated with CsA alone or CS alone (Figure 3A) after the first remission was induced. Among the 23 patients in CsA alone group, the estimated median RFS was 82 months, with a median observation period of 34 months (range, 1 to 126 months). On the other hand, among the nine patients in the group treated with CS alone, the estimated median RFS was 9 months, with a median observation period of 7 months (range, 3 to 46 months). The duration of initial remission was, therefore, longer after CsA than after CS, and the differences was statistically significant ($p<0.0001$).

Exposure to other agents might affect the efficacy of CsA to sustain remission. Figure 3B illustrates the duration of RFS among patients in the CsA-containing group (Figure 1, A1 plus A2) and the CS-group (Figure 1, B1 plus B2) after the first remission had been induced. Among the 41 patients in the CsA-containing group, the estimated median RFS was 103 months, with a median observation period of 45 months (range, 1 to 196 months). On the other hand, among the 15 patients in the CS group, the estimated median RFS was 33 months, with a median observation period of 9 months (range, 1 to 55 months). The group of patients who achieved remission with a CsA-containing regimen had a longer duration of initial remission in comparison to the CS group, with the difference being statistically significant ($p<0.01$). There was no difference in the age at onset between the CsA-containing group and CS group, which was 54±18 years old (mean±SD), with a range from 18 to 82 and 56±20 years old with a range from 18 to 89, respectively. It was difficult to derive any conclusions on RFS in the cyclophosphamide group because there were only two patients in this group.

Factors related to first relapse

Twenty-four out of 58 patients (41%) have had at least one relapse (Figure 1). Fifteen out of these 24 relapsed patients were in the CsA-containing group (Figure 1A). When the rate of first relapse was evaluated in relation to maintenance CsA therapy, it was found that of the 14 patients whose CsA was discontinued, 12 (86%) relapsed after a median period of 3 months (range, 1.5 to 40 months), while only 3 of 27 patients (11%) relapsed during maintenance therapy (Figure 1A). This indicates that maintenance CsA therapy prevents relapse ($p<0.001$, χ^2 test). The other agents used for remission induction might have affected the efficacy of CsA as maintenance therapy. However, the efficacy of CsA at preventing relapse was also noted in the patients who were treated with CsA alone (Figure 1-A1) ($p<0.01$) as well as in the patients who were treated with CsA and the other agents (Figure 1-A2) ($p<0.05$). In contrast, 8/15 patients in the CS group (53%) relapsed within 2 to 40 months after remission and 7/8 patients (88%) relapsed during maintenance prednisolone therapy, thus suggesting the difficulty of maintaining remission with prednisolone.

Relapse-free period after discontinuation of CsA

The relapse-free period after discontinuation of CsA therapy (shown as RFS2 in Figure 1) was 10±14 months (n=10), with a range of 1.5 to 40 months, indicating that

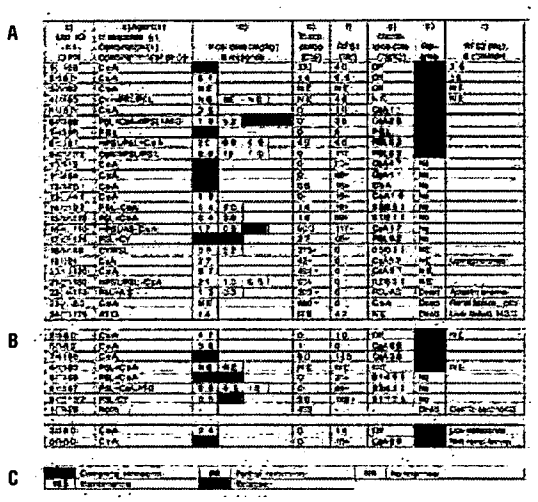

Figure 4 (*left*). Patients in first relapse (A), second relapse (B) and third relapse (C). Abbreviations in each column are the same as those shown in the legend to Figure 1 except for a) list #2; list number in this figure followed by the list number shown in Figure 1 (#1) and UPN, g) ᵗdoses of prednisolone/CsA in order, ᶜdoses of cyclophosphamide/prednisolone in order, §doses of prednisolone/cyclophosphamide in order, i) MN; membranous nephropathy, HBV; hepatitis B virus infection.

relapse can occur even 3 years after the discontinuation of CsA. Two patients have maintained remission after discontinuation of CsA therapy (Figures 1A, 9-26 and 10-19); however, the relapse-free periods after discontinuation of CsA therapy are only 1 and 5 months.

Duration of CsA therapy

The mean duration of CsA therapy in patients who relapsed after discontinuation of CsA was 76±32 months, with a range of 10 to 108 months (n=12). In contrast, the mean duration of CsA therapy in patients who are in remission under CsA therapy was 45±48 months (n=24), with a range of 1 to 192 months. The mean dosage of CsA in patients who are in continuing remission for more than 24 months was 2.2±0.8 mg/kg (n=10), 40% of the beginning dose, with a range from 1.1 to 3.8 mg/kg (Figure 1A), excluding one patient (23-130) whose dose of CsA had gradually been increased.

Response of patients in first relapse to different therapies

All patients who had a first relapse were re-treated in an attempt to re-induce remission, and this treatment was successful in 18/24 patients (75%) (Figure 4A, 1-26-28 to 17-46-124 and 24-28-129; corresponding to list No (#2) in Figure 4-list No(#1) in Figure 1-UPN in order). In the 15 relapsed patients in the CsA-containing group, CsA alone was again tried as the initial re-induction therapy for 11 patients, and this treatment was successful in eight of these 11 patients (73%). Three patients did not respond to CsA; one patient with low adherence (frequent self-discontinuation of CsA) (19-7-21), one patient whose dose of CsA was low due to renal dysfunction associated with membranous nephropathy (23-27-80), and one patient who seemed to be resistant to CsA (20-11-120). The remaining four patients were retreated by sequential administration of immuran and ATG (24-28-129), CS concomitantly with anabolic steroids (22-25-179) or CsA (6-29-68), or CS in combination later on with CsA (21-26-160). The two patients treated with ATG (24-28-129) or with a combination of CS and CsA (21-26-160) responded to therapy.

In the eight relapsed patients in the CS group, CS was again tried as an initial re-induction therapy for six patients. CS alone was again tried as the initial re-induction therapy for two patients, and this treatment was successful (7-43-96, 14-44-27). Three patients responded to a combination of CS and CsA (8-45-67, 15-52-178) or CS and cyclophosphamide (17-46-124). One patient responded to CsA (9-42-132). The remaining two patients failed to respond to cyclophosphamide (4-53-93) or methylprednisolone followed by anabolic steroids (16-47-110), but responded to CS and CsA, respectively. As a result, 8/8 relapsed patients in CS responders achieved remission and CsA or cyclophosphamide was newly introduced in 4/8 patients as maintenance therapy. A combination of cyclophosphamide and CS was tried for one relapsed patient (18-57-49) in the cyclophosphamide group but this patient remained transfusion-dependent. Three patients were lost to the follow-up after successful re-induction (3-8-182 and 17-46-124) or during re-induction therapy (21-26-160).

Recurrent relapses

A second relapse occurred in 9/17 patients (Figure 4A). Three out of nine patients experienced a second relapse after discontinuation of CsA therapy (1-4-28, 2-5-60 and 3-8-182). One patient was lost to the follow-up (3-8-182). Seven out of the remaining eight patients were re-induced to a third remission (Figure 4B). One patient was treated by transfusion alone because of the presence of gastric carcinoma (1-4-28). CsA with or without concomitant CS was tried in 6/7 patients and induced remission in all six patients. One patient who had responded to CS achieved complete remission with a later on combination of cyclophosphamide (9-42-132). Thus, no patient treated with CS alone was present after the second relapse. A third relapse occurred in 4/7 patients (Figure 4B). One patient autonomously decided to discontinue CsA and relapsed (2-5-60). Two patients were lost to the follow-up after the third relapse (4-53-93 & 7-43-96). The remaining two patients were successfully re-induced into remission by CsA alone (Figure 4C) but have been experiencing frequent relapses up to the present due to self-discontinuation of CsA (2-5-60) and the limitation of dose escalation due to mild renal failure (5-6-50).

Mortality and overall survival (OS)

Six out of 62 patients (9.7%) died and the estimated 10-year OS after the onset of PRCA was 95%; the median OS

has not yet been reached. Two patients did not respond to remission induction therapy and died from infections (Figure 1D, 60-138 and 62-158). After the first relapse, three patients in the CsA-containing group died (Figure 4A, 22-25-179, 23-27-80 and 24-28-129). One patient (22-25-179) eventually developed aplastic anemia and died from a serious infection, one patient (23-27-80) died from renal failure associated with membranous nephropathy, and the other (24-28-129) died due to liver failure caused by cirrhosis of the liver after hepatitis B virus infection. After a second relapse, one patient (Figure 4B, 1-4-28) in the CsA-containing group, died; the cause of death was gastric carcinoma found 4 years after the onset of PRCA. All four of these patients were in the CsA-containing group who had experienced relapse at least once; however, the OS was not significantly different between patients in the CsA-containing group and those in the CS group (p=0.104).

Discussion

Primary idiopathic PRCA is a clinical disorder defined by the absence of any other disease and is pathogenetically heterogeneous. The most frequent disease underlying secondary PRCA is large granular lymphocyte leukemia (LGL),[6] also referred to as lymphoproliferative disease of granular lymphocytes[13] or granular lymphocyte proliferative disorders.[14] This often has unique clinical features such as autoimmune diseases including rheumatoid arthritis, aplastic anemia, PRCA, neutropenia and thrombocytopenia, and sustained remission may be achieved by treatment with CsA or cyclophosphamide, with or without prednisolone.[6,13,14] The diagnosis of LGL is somewhat difficult in patients without lymphocytosis. Although 14/185 patients were classified as having LGL and secondary PRCA in this study, it remains possible that some patients with LGL are included in this series of cases with supposedly primary idiopathic PRCA. In addition, the data of the current study are derived from a retrospective analysis and the responses cannot be attributed to CsA alone but must, more appropriately, be attributed to CsA-containing regimens, which include both CsA alone and CsA plus other drugs. In this study, we showed, for the first time, that the median RFS of patients in the CsA-containing group was 103 months, which is longer than that seen in the CS group (33 months) (p<0.01). In the CsA-containing group, the discontinuation of CsA was strongly correlated with relapse (p<0.001). Two patients have maintained remission after the discontinuation of CsA; however, the relapse-free periods after the discontinuation are only 1 and 5 months. Considering that a relapse can occur even 40 months after the discontinuation of CsA, these observation periods may be insufficient to conclude that some patients can be cured by CsA. In contrast, 88% of the relapses in the CS group occurred during maintenance prednisolone therapy. Therefore, CsA-containing therapy can sustain a longer duration of initial remission than CS and seems to be important to prevent relapse. Although vigorous and continuous immunosuppressive treatment is capable of inducing and maintaining remission in a majority of patients, it carries an increased risk of serious infections,[15] malignancy,[17,18] and sterility.[19] In our series, two patients died during remission induction due to opportunistic infections (*Pneumocystis jiroveci* pneumonia and bacterial meningitis), which suggests that adequate prevention and treatment of infection are requisites for successful management of patients. After achieving the first remission, four patients died and all of them were CsA responders who relapsed at least once. The causes of death were the development of aplastic anemia, renal failure with membranous nephropathy, liver failure associated with hepatitis B virus infection and gastric carcinoma. The relationship of CsA with the former three diseases is unclear because CsA is one of the effective treatments for aplastic anemia,[20] membranous nephropathy[21] and probably for hepatitis B virus infection as well.[22] Although immunosuppressive therapy enhances viral replication, it has been shown that CsA by itself impairs hepatitis B virus replication by blocking cytosolic calcium signaling.[22] Gastric carcinoma was found in one patient 4 years after the onset of PRCA, but the relationship of this neoplasm to the pathogenesis of PRCA or its treatment with CsA is not clear. Organ transplant experiences have shown that long-term immunosuppression is associated with post-transplant malignancies.[18,19] Therefore, continuous and careful follow-up is required for patients receiving long-term CsA therapy. In addition, the mean maintenance dosage of CsA in Japanese patients who are continuing in first remission for more than 24 months was 2.2±0.8 mg/kg, 40% of the initial dose, suggesting that it would be difficult to reduce the dose of CsA under this level while maintaining remission. One important question is whether or not the maintenance of patients in remission may have a beneficial influence on survival. In the era when CsA was not yet available, Clark *et al.* showed that the treatment of relapses was almost equally successful in 10/13 patients entering a second or third remission, and that the median survival of patients with primary PRCA was 14 years.[11] In our cohort the estimated 10-year OS was 95% and the median OS has not yet been reached; furthermore, we found that CsA-containing regimens can sustain remission for more than 10 years as continuous maintenance therapy. The decreased probability of relapse and the resulting decreased requirement of blood transfusions reduces the dangers of hemolysis, infections and iron overload with possible superoxide damage to body tissues. Although CsA-containing regimens are more expensive than prednisolone, CsA-containing regimens seem to be important to prevent relapse.

In conclusion, we have demonstrated for the first time that CsA-containing regimens, in comparison to CS, sustain a more prolonged RFS in patients with primary idiopathic PRCA. Furthermore, maintenance CsA-containing regimens seem to be important to prevent relapse. Nevertheless, an individualized approach to the manage-

ment of primary PRCA is suggested, and other therapeutic modalities may be required to cure primary PRCA. Prospective randomized studies are needed to identify agents and/or strategies that can cure primary idiopathic PRCA and to determine whether or not maintenance treatment is necessary. It should be appreciated that such studies must last decades considering the recurrent nature of this disorder.

Appendix

The following institutions participated in the Collaborative Study Group: Aichi Medical School, Akita University, Asahikawa Medical School, Chiba University, Dokkyo Medical School, Ehime University, Fujita Health University, Fukui University, Fukui National Hospital, Fukuoka University, Fukushima Medical University, Gifu University, Gunma University, Hamamatsu Medical School, Hirosaki University, Hiroshima University, Hokkaido University, Hyogo Medical University, Iwate Medical School, Jichi Medical School, Jikei University, Juntendo University, Kagawa Childrens' Hospital, Kagawa University, Kagoshima University, Kanazawa University, Kanazawa Medical School, Kansai Medical University, Kawasaki Medical School, Keio University, Kinki University, Kitazato University, Kobe University, Kochi University, Kumamoto University, Kurume University, Kyoto Prefectural University, Kyoto University, Kumamoto Medical Center, Kyushu University, Mie University, Nagasaki University, Nagoya City University, Nagoya Medical Center, Nagoya University, Nara Medical University, National Cancer Center, National Institute of Infectious Diseases, Niigata University, Nishi Sapporo National Hospital, Nippon Medical School, Nippon University, NTT Kanto Medical Center, Oita University, Okayama Medical Center, Okayama University, Osaka City University, Osaka Medical School, Osaka National Hospital, Osaka University, Ryukyu University, Saga University, Saitama Medical School, Sapporo Medical School, Sendai Medical Center, Shimane University, Shinsyu University, Showa University, St. Marianna University, Teikyo University, Toho University, Tohoku University, Tokai University, Tokushima University, Tottori University, Tokyo Medical Center, Tokyo Medical School, Tokyo Medical and Dental University, Tokyo University, Tokyo Women's Medical School, Tsukuba University, University of Occupational and Environmental Health, Wakayama Medical University, Waseda University, Yamagata University, Yamaguchi University, Yamanashi University, Yokohama City University.

Authors' Contributions

KS: designed the research, analyzed the data and wrote the paper. MH: analyzed data and contributed to writing the paper. NF: analyzed data. MT, MB, KD, HT, SN, AU, MO, and KO: designed the research and contributed to the organization of this collaborative study.

Conflict of Interest

The authors reported no potential conflicts of interest.

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Long-term response and outcome following immunosuppressive therapy in thymoma-associated pure red cell aplasia: a nationwide cohort study in Japan by the PRCA collaborative study group

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ABSTRACT

Background
Thymoma-associated pure red cell aplasia (PRCA) accounts for a significant proportion of cases of secondary PRCA and immunosuppressive therapy has been reported to be useful in this condition. However, because of its rarity, the long-term response and relapse rates after immunosuppressive therapy are largely unknown, and optimal management of this disorder remains unclear. The aim of this study was to collect more information on the outcome of patients with thymoma-associated PRCA.

Design and Methods
We conducted a nationwide survey in Japan. From a total of 185 patients, comprising 73 with idiopathic and 112 with secondary PRCA, 41 patients with thymoma were evaluated for this report. End-points of this study were the response rate, duration of the response after immunosuppressive therapy and overall survival.

Results
Surgical removal of thymoma was reported in 36 patients, 16 of whom developed PRCA at a median of 80 months post-thymectomy. First remission induction therapy was effective in 19 of 20 patients treated with cyclosporine, 6 of 13 patients treated with corticosteroids and 1 of 1 treated with cyclophosphamide. No cyclosporine-responders relapsed within a median observation period of 18 months (range; 1 to 118 months). Relapse of anemia was observed in three corticosteroid-responders who did not receive additional cyclosporine. Only two patients were in remission after stopping therapy for 19 and 67 months. The estimated median overall survival time of all patients was 142 months.

Conclusions
Thymoma-associated PRCA showed an excellent response to cyclosporine and cyclosporine-containing regimens were effective in preventing relapse of anemia. It does, however, remain uncertain whether cyclosporine can induce a maintenance-free hematologic response.

Key words: pure red cell aplasia, thymoma, cyclosporine.

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Introduction

Acquired pure red cell aplasia (PRCA) is an anemic condition characterized by the absence of reticulocytes in blood and the absence of erythroid precursors in the bone marrow.[1-4] Other hematopoietic cell lineages are present with no evident morphological abnormalities. Secondary PRCA is associated with various underlying diseases including lymphoproliferative disorders, thymoma, solid tumors, autoimmune diseases, drugs and viral infections.[1,2] The association of PRCA with thymoma was first described in 1928 by Matras and Priesel,[1] and thymoma-associated PRCA accounts for a significant proportion of the secondary cases.[5,6] Thymoma-associated PRCA is generally thought to be an organ-specific autoimmune disease as well as an idiopathic form, and immunosuppressive therapy, including corticosteroids, cyclophosphamide and cyclosporine, has been reported to be useful.[5,7,8,9] Thompson *et al.* recently reported their 50-year single institution experience with 13 patients with thymoma-associated PRCA,[10] and showed that surgical resection of the thymoma was insufficient to induce normalization of erythropoiesis, and that anti-thymocyte globulin was an effective adjuvant treatment but associated with high treatment-related morbidity due to frequent infectious complications. However, the optimal management of thymoma-associated PRCA and the long-term outcome after immunosuppressive therapy remain unclear because of the rarity of this disorder.

The efficacy and long-term outcome after immunosuppressive therapy for secondary PRCA could differ according to the underlying diseases. To date, the overall long-term response and relapse rates after immunosuppressive therapy in acquired PRCA are largely unknown. We, therefore, conducted a nationwide survey to investigate the current status of immunosuppressive therapy for acquired chronic PRCA based on a relatively large cohort of patients in Japan. This report is a summary focusing on immunosuppressive therapy for thymoma-associated PRCA.

Design and Methods

Data collection and patients' characteristics

The first questionnaires were sent to 109 hematology departments in Japan to estimate the number of patients aged 15 and above who had been newly diagnosed as having acquired PRCA between 1990 and 2006. Patients with human parvovirus B19 infection-associated PRCA were excluded. Eligible patients were limited to those who had been diagnosed during this period in order to minimize the effect of transfusion-associated hepatitis C virus infection. Overall, 273 patients were enrolled from 45 institutions. Secondary questionnaires were then sent to these institutions to collect data regarding underlying diseases, laboratory findings including peripheral blood cell counts and leukocyte differentials, results of bone marrow examination, immunological and cytogenetic parameters, efficacy of immunosuppressive therapy and outcome. Morphological diagnosis of bone marrow was done by hematologists at each institution. Of the 185 patients identified, 73 patients were classified as having idiopathic PRCA and 112 as having secondary PRCA.

Table 1. Co-morbidity in patients with thymoma-associated PRCA (n=41).

Underlying diseases	*Number of patients*
Autoimmune disease	11
Myasthenia gravis	6
Systemic lupus erythematosus	1
Mixed connective tissue disease	1
Dermatomyositis	1
Polyneuropathy	1
Autoimmune hemolytic anemia	1
Malignancy	5
Myelodysplastic syndrome	1
Stomach	1
Breast	1
Thyroid	1
Bladder	1

The classification of PRCA was based on the criteria proposed by the Hematopoietic Organs Research Committee of the Ministry of Health, Labor and Welfare of Japan in 2005.[11] This classification was fundamentally based on the criteria proposed by Dessypris and Lipton.[2] Forty-two patients had both thymoma and PRCA. One patient who had undergone autologous hematopoietic stem cell transplantation for recurrent malignant thymoma before the onset of PRCA was excluded from this study, so 41 patients were finally selected for analysis of thymoma-associated PRCA. Personal identifying information was protected by giving each data set a unique patient number at each participating institution. This study was approved by the institutional review board, and performed according to the Declaration of Helsinki and the Ethical Guidelines for Epidemiological Research of the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare of Japan.

The age of the patients at the onset of PRCA ranged from 27 to 82 years (median age, 66 years) and there was a 3:4 male to female ratio of cases. Autoimmune diseases and malignancies were complications in 11 and five patients, respectively (Table 1). Thymoma histology was varied with one case of type A, nine type AB, three type B1, and four type B2 cases according to the WHO classification of histological typing of tumors of the thymus.[12] Hyperplasia was reported in one case, and histological subtypes could not be determined in 23 cases. The hemoglobin concentration ranged from 2.7 to 10.9 g/dL with a median of 5.8 g/dL.