Treatment of aplastic anemia with rabbit antithymocyte globulin as first-line immnosuppressive therapy: A single-center retrospective study

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Objective: The aim of this study was to reveal the efficacy and safety of rabbit antithymocyte globulin (rATG) in patients with aplastic anemia (AA).

Patients: This study included 17 consecutive patients in Tenri Hospital from May 2009 through November 2013 who were diagnosed with AA and first treated with rATG in combination with or without cyclosporine (CS), including one who had been previously treated with CS alone.

Results: Ages ranged from 8 to 81, with a median of 68. The diseases included 5 non-severe (NSAA), 7 severe (SAA), and 5 very severe AA (VSAA). Paroxysmal nocturnal hemoglobinuria (PNH)-type cells were present in 11. Rabbit ATG was administered at a dose of 2.5 or 3.75 mg/kg/day. Cyclosporine was concurrently given to all but one. At 6 months, 10 (71%) of 14 evaluable patients achieved a partial response (PR) and 2 finally fulfilled the criteria for a complete response. Nine (75%) of the 12 NSAA/SAA patients achieved a PR or more, while the hematological response in 3 of 4 VSAA patients at >6 months did not reach the level of PR. The percentage of PNH-type cells showed no significant change after treatment. Three patients aged ≥75 years old died, including 2 with unexpected cardiopulmonary arrest. With a median follow-up of 504 days, the overall survival rate at 2 years was 79%.

Conclusion: This study indicates that rATG is the treatment of choice for NSAA/SAA patients who are ineligible for allogeneic transplantation. However, we should be cautious when using rATG for aged patients.

Keywords: aplastic anemia, rabbit antithymocyte globulin, elderly patients, paroxysmal nocturnal hemoglobinuria-type cells

INTRODUCTION

Aplastic anemia (AA) is characterized by bone marrow hypoplasia and pancytopenia, and immune mechanisms leading to the destruction of hematopoietic stem cells have been suggested for the development of the disease.1 Horse antithymocyte globulin (hATG), polyclonal antibodies generated in horses immunized with human thymocytes, was previously the standard treatment for patients with AA who were ineligible for allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donors.1,2
The combination of hATG and cyclosporine (CS) with or without other immunosuppressive drugs for AA led to a hematological response rate of 62 to 70% and long-term survival of 58 to 93%. In 2009, hATG was withdrawn in Japan and many other countries worldwide, but not in the United States, and rabbit ATG (rATG), which was initially used for patients who relapsed or were refractory to the first course of hATG, is currently the only available preparation of ATG in Japan. Retrospective and prospective studies evaluating rATG as first-line immunosuppressive therapy for AA have indicated that the response rate at 6 months ranges from 34 to 64%, and the overall survival rate at 2 or 3 years ranges from 55 to 94%. Thus, although rATG is more immunosuppressive than hATG, the treatment results with rATG are not necessarily superior to those with hATG, and a randomized study comparing rATG and hATG as the first treatment for AA revealed the inferiority of rATG in terms of both hematological response and survival.

Few studies have addressed the efficacy and safety of rATG in Japanese patients with AA. Here, we report the treatment results for a total of 17 patients in our hospital, including one pediatric patient reported previously, who were diagnosed with AA and treated with rATG in combination with or without CS as the first-line therapy.

PATIENTS AND METHODS

Patients

This study included 17 consecutive patients in Tenri Hospital from May 2009 through November 2013 who were diagnosed with AA and first treated with rATG in combination with or without CS, including one who had been previously treated with CS alone. The diagnosis of AA was made by complete blood count and bone marrow examination. The count met at least two of the following parameters: i) hemoglobin level <10 g/dL, ii) platelet count <50 × 10^3/μL, and iii) neutrophil count <1.5 × 10^3/μL. Bone marrow hypocellularity was determined by the examination of marrow fragments obtained by aspiration and/or biopsy specimens. Cytogenetic analysis was performed by standard G-banding preparation. The diseases were categorized into non-severe AA (NSAA), severe AA (SAA), and very severe AA (VSAA): SAA, bone marrow cellularity of <30% and at least two of the following parameters: neutrophil count <0.5 × 10^3/μL, platelet count <20 × 10^3/μL, and reticulocyte count <20 × 10^3/μL; VSAA, as for SAA but neutrophil count <0.2 × 10^3/μL; and NSAA, not fulfilling the criteria for SAA or VSAA. The VSAA, SAA, and NSAA categories correspond respectively to the severity stages of 5, 4, and 1-3, determined by the Guidelines for the Diagnosis and Treatment of Intractable Anemia in Japan.

Flow Cytometry Detecting Paroxysmal Nocturnal Hemoglobinuria (PNH)-type Cells

We applied high-sensitivity two-color flow cytometry to detect and quantify the PNH-type cells. Briefly, a blood sample was diluted 1:100 with phosphate-buffered saline (PBS)-albumin for red cell analysis, while granulocytes were enriched with dextran/saline and resuspended in PBS-albumin. The FITC-CD55 (IM2725; Beckman Coulter) and FITC-CD59 (IM3457U; Beckman Coulter) antibody cocktail was combined with PE-CD235a (R7078; Dako) or PE-CD11b (Leu-15; Beckton Dickinson) monoclonal antibody and incubated with cell samples for 30 min at 4°C. Red cells or granulocytes were initially identified on the basis of an SSC-FSC scattergram. A total of >500 × 10^3 CD235a-positive cells or >250 × 10^3 CD11b-positive cells were analyzed for CD55/CD59 expression. The cut-off values for the presence of PNH-type cells were 0.005% for CD55(−)/CD59(−) red cells and 0.003% for CD55(−)/CD59(−) granulocytes.

Treatment Protocol

Rabbit ATG (Thymoglobulin, Sanofi) was administered as a daily intravenous infusion over 12 hours at a dose of 3.75 mg/kg for 5 days in 11 patients and 2.5 mg/kg for 5 days in 6; the dosage was left to the discretion of the
treatment physician. For 16 patients, oral CS (Neoral, Novartis Pharma) was started at an initial dose of 5 to 6 mg/kg per day on the first day of rATG and continued for at least 6 months, aiming at a trough CS blood level of 150 to 250 ng/mL. One patient with NSAA was treated with rATG alone. To prevent an immediate allergic reaction against rATG, methylprednisolone was administered intravenously at a dose of 2 mg/kg from days 1 to 5, and an antihistamine (diphenhydramine) and antipyretic agent (acetaminophen) were given before each infusion of rATG. On day 6, oral prednisolone at a dose of 1 mg/kg was started, and tapered and stopped appropriately; the dosage of prednisolone was adjusted according to the development and severity of serum sickness. Transfusions of red cells or platelets were carried out to maintain a hemoglobin level of 7 g/dL and platelet level of 20 × 10^3/μL. Granulocyte-colony stimulating factor (G-CSF) was given when the neutrophil count was <0.5 × 10^3/μL. Prophylactic antifungals (itraconazole, 200 mg QD, or fluconazole, 100 mg QD) and an antiviral (acyclovir, 200 mg QD) were given orally, and levofloxacin (500 mg QD) was given when the neutrophil count was <0.2 × 10^3/μL. Active cytomegalovirus (CMV) infection was monitored by the CMV pp65 antigenemia assay. All patients were hospitalized from the start of rATG through the period when serum sickness can develop. All patients were informed of both the benefits and the complications of rATG ± CS, and consented to the treatment.

Response and Statistical Analysis

The hematological response was defined according to the criteria of Camitta. Briefly, a complete response (CR) for SAA/VSAAS was defined as transfusion independence associated with a normal hemoglobin level for the age, neutrophil count of >1.5 × 10^3/μL, and a platelet count of >150 × 10^3/μL; a partial response (PR) was defined as no longer meeting the criteria for SAA and no transfusion dependence. A partial response for NSAA was defined as transfusion independence or meeting the levels of hemoglobin, neutrophils, and platelets as defined by the criteria. Responses were confirmed by two or more blood counts at least 4 weeks apart and measured when not receiving G-CSF. Assessment of the response was performed at 3, 6, 12, and 18 months after the rATG treatment. The length of survival was investigated using the Kaplan-Meier method.

RESULTS

Patients’ Characteristics

Table 1. Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>17</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>68 (8 to 81)</td>
</tr>
<tr>
<td>Number of &gt;60-year-old patients</td>
<td>13</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>8 / 9</td>
</tr>
<tr>
<td>Disease severity: NSAA/SAA/VSAAS</td>
<td>5 / 7 / 5</td>
</tr>
<tr>
<td>Presence of PNH-type cells</td>
<td>11 (NSAA, 3; SAA, 5; VSAAS, 3)</td>
</tr>
<tr>
<td>Median hemoglobin level (g/dL) (range)</td>
<td>6.0 (3.7 to 8.8)</td>
</tr>
<tr>
<td>Median reticulocyte count (x 10^3/μL) (range)</td>
<td>18.5 (4.7 to 43.2)</td>
</tr>
<tr>
<td>Median neutrophil count (x 10^3/μL) (range)</td>
<td>0.5 (&lt;0.1 to 1.3)</td>
</tr>
<tr>
<td>Median platelet count (x 10^3/μL) (range)</td>
<td>8.0 (2.0 to 18.0)</td>
</tr>
<tr>
<td>Previous treatment</td>
<td>1 (cyclosporine alone)</td>
</tr>
<tr>
<td>Median duration from initial presentation to rATG (days) (range)</td>
<td>28 (8 to 1047)</td>
</tr>
</tbody>
</table>

NSAA, non-severe aplastic anemia; SAA, severe aplastic anemia; VSAAS, very severe aplastic anemia; PNH, paroxysmal nocturnal hemoglobinuria
enty-six percent of the patients were aged 60 years or older. No apparent cause of the disease was identified in any patient. All 5 NSAA patients were transfusion-dependent. No patient had cytogenetic abnormality recognized by conventional G-banding and morphological abnormality of hematopoietic cells in the bone marrow. PNH-type cells were detected in 11 patients, comprising 0.014 to 5.082% red cells and 0.023 to 2.305% granulocytes (Figure 1). Of the 11 patients, two showed low levels of serum haptoglobin and one showed an elevated lactate dehydrogenase level. Nevertheless, there was no clinical evidence of PNH. The presence of PNH-type cells was not related to disease severity. No patient had comorbidities that prevented the use of rATG and additional drugs.

**Hematological Response**

The course of the hematological response of individual patients is illustrated in Figure 2. At 3 months after the treatment of rATG, 4 (29%) of 14 evaluable patients achieved PR, while 10 (71%) of 14 patients achieved the response at 6 months. Of 10 patients who were followed for over 12 months, 2 finally fulfilled the criteria of CR. All ≥PR patients achieved the response within 6 months. The response rates of SAA/NSAA patients at 3, 6, 12, and 18 months were 30% (3 of 10), 90% (9 of 10), 100% (8 of 8), and 100% (7 of 7), respectively. In contrast, the hematological response in 3 of 4 VSAA patients followed for over 6 months did not reach the level of PR. There was no significant difference between responders (n = 10) and non-responders (n = 4) at 6 months of rATG in age <60 vs. ≥60, male vs. female, presence vs. absence of PNH-type cells, the dose of rATG 2.5 vs. 3.75 mg/kg, and the duration from initial presentation to rATG treatment (Table 2). Reflecting on the fact that the non-responder group included 3 VSAA patients (Figure 2), pretreatment blood counts tended to be lower than those of the responder group (Table 2).

Figure 3 shows the course of increases in blood counts

![Figure 1](example.png)

**Figure 1.** Example of high-sensitivity flow cytometry analysis of red blood cells (top) and granulocytes (bottom) with PNH phenotype before and after rATG treatment (case no. 1).
in 9 patients who achieved ≥PR and were followed for over 12 months, consisting of 3 NSAA, 5 SAA, and 1 VSAA patient. The numbers of reticulocytes, neutrophils, and platelets significantly increased at 3 months, while the increase of the hemoglobin level was significant at 6 months. Next, we compared the proportion of PNH-type cells in 7 patients who had PNH-type cells before treatment and achieved ≥PR after treatment. As shown in Figures 1 and 4, the percentages of CD55(−)/CD59(−) red cells and CD55(−)/CD59(−) granulocytes in the peripheral blood showed no significant change after treatment.

**Morbidity, Mortality, and Survival**

Infectious complications included febrile neutropenia in 5 patients, CMV antigenemia in 6, and pulmonary aspergillosis in 2. One patient developed the transient proliferation of plasmacytoid cells in the blood, which was associated with reactivation of the Epstein-Barr virus.

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Table 2. Comparison between responders and non-responders at 6 months of rATG treatment

<table>
<thead>
<tr>
<th>Pretreatment blood counts (median)</th>
<th>Responders (n = 10)</th>
<th>Non-responders (n = 4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>≥60</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&lt;60</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>PNH-type cells</td>
<td>Present</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>ATG dosage</td>
<td>2.5 mg/kg/day</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3.75 mg/kg/day</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Infectious complications</td>
<td>Present</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Pretreatment blood counts (median)</td>
<td>Hemoglobin (g/dL)</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Reticulocytes (x 10^3/µL)</td>
<td>20.4</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Neutrophils (x 10^3/µL)</td>
<td>0.66</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Platelets (x 10^3/µL)</td>
<td>9.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Duration from initial presentation to rATG</td>
<td>Median (days)</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Range (days)</td>
<td>12 to 1,047</td>
<td>8 to 29</td>
</tr>
</tbody>
</table>

*Fisher’s exact test. **Unpaired t-test.
Figure 3. Increases in the level of hemoglobin (g/dL), number of reticulocytes (×10³/µL), number of neutrophils (×10³/µL), and number of platelets (×10³/µL) in 9 patients who showed >PR and were followed for >12 months. The mean values ± SE at 3, 6, and 12 months after rATG are shown. The differences were evaluated by the paired t-test, and the P-value of each comparison is indicated.

Figure 4. Changes in the proportions of PNH-type red blood cells (A) and granulocytes (B) in the blood of 7 patients who harbored PNH-type cells before treatment and showed PR or more after treatment. The difference was evaluated by the paired t-test.

Table 3. Three deaths after rATG treatment

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at diagnosis of AA</th>
<th>Severity</th>
<th>Days after rATG</th>
<th>Response at death</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>75</td>
<td>NSAA</td>
<td>40</td>
<td>NR</td>
<td>CPAOA</td>
</tr>
<tr>
<td>9</td>
<td>78</td>
<td>SAA</td>
<td>189</td>
<td>NR</td>
<td>CPAOA</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>VSAA</td>
<td>193</td>
<td>NR</td>
<td>Pneumonia</td>
</tr>
</tbody>
</table>

CPAOA: cardiopulmonary arrest on arrival; NR, no response
Serum sickness was seen in 3 patients on days 10 to 40; their symptoms included polyarthralgia in 3, skin rash in 1, fever in 1, and leg edema in 1. A total of 3 patients died, all of whom were ≥75 years old and lacked a response to treatment (Table 3). With a median follow-up period of 504 days (range, 35 to 1,563 days), the overall survival rate at 2 years was 79% for all patients and 83% for patients with SAA (Figures 5A and B). No ≥PR patient showed relapsed AA. There was no evolution to other clonal hematologic diseases.

DISCUSSION

Here, we have reported the response to rATG ± CS and survival in a total of 17 patients with AA who were diagnosed and treated in our institution. Ten (71%) of 14 patients achieved PR at 6 months and the estimated survival rate at 2 years was 79%. These treatment results are comparable to or even better than those of earlier studies.7,9-15 The favorable results might be due, in part, to the fact that our study included 11 (65%) patients who harbored PNH-type cells, as the presence of a minor PNH clone population has been shown to correlate with a better response to immunosuppressive treatment and longer survival among patients with AA.15,22-24 The “immune escape theory” states that PNH-type stem cells acquire a survival advantage over non-PNH-type stem cells when the immune mechanism targets hematopoietic stem cells, so that immunosuppressive treatment theoretically leads to the expansion of normal stem cells more than PNH-type stem cells.25-27 However, in a series of AA patients treated with immunosuppressive therapy, the proportions of PNH-type granulocytes remained constant in those who harbored PNH-type cells and responded to the therapy.22 In line with this, our results showed that the proportion of PNH-type cells was not significantly affected by immunosuppressive treatment; therefore, absolute numbers of PNH-type and normal blood cells were considered to increase concurrently in response to immunosuppressive therapy. Thus, the “immune escape theory” is not necessarily supported by clinical observation. Sugimori et al. suggested that immune attack against hematopoietic stem cells that allows clonal expansion of PNH-type cells may occur only at the early stage of AA.22 At present, the causal relationship between the presence of PNH-type cells and a more favorable response to immunosuppressive therapy remains to be elucidated.

Another important point to note is that rATG ± CS was well tolerated and the regimen was safely completed in all patients, even though the majority of our cohort consisted of elderly patients and the median age was 68 years (Table 1). Infectious complications were readily resolved, apart from one death from pneumonia, with the treatment of febrile neutropenia according to the guidelines and appropriate intravenous antibiotics, antifungals, and antivirals. The prophylactic use of acyclovir and itraconazole/fluconazole and careful monitoring of CMV antigenemia are likely to have contributed to the low in-
fectious mortality. Thus, with the knowledge of potential complications of immunosuppressive treatment and the supportive treatment for infectious and bleeding comorbidity, rATG ± CS is the treatment of choice for the majority of patients with AA who are not eligible for allogeneic transplantation from HLA-matched sibling donors. Nevertheless, although there is no upper age limit for ATG treatment, we should be cautious when using rATG for aged patients, as we encountered 3 deaths of ≥75-year-old patients with a lack of response to immunosuppressive treatment. Unexpected cardiopulmonary arrest observed in two such patients (Table 3) could be included in serious cardiac events after ATG, which was reported in elderly patients treated with this immunosuppressive drug.

In contrast to the favorable response to rATG ± CS in patients with NSAA/SAA, 3 of 4 VSAA patients followed over 6 months did not respond to the treatment and 2 patients (nos. 12 and 14) required long-term transfusions of both red cells and platelets for persistent cytopenia. Although there is no second-line treatment approved for refractory disease, eltrombopag, a synthetic small-molecule thrombopoietin agonist, was very recently shown to be effective in a fraction of patients with AA who were refractory to the initial immunosuppressive treatment. The study showed that 11 (44%) of 25 such patients had a hematologic response, including 9 patients involving the platelet counts, 6 involving the hemoglobin levels, and 9 involving the neutrophil counts, suggesting that thrombopoietin may not only stimulate the production and differentiation of megakaryocytes but also increase the number of hematopoietic stem and progenitor cells. Of course, to introduce the new drug into clinical practice, further clinical trials that confirm the efficacy and determine the optimal treatment schedule are required.

REFERENCES


ウサギ抗胸腺細胞グロブリンを用いた再生不良性贫血の治療: 当院における治療成績

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目的: 現在、再生不良性貧血(aplastic anemia; AA)の薬物治療として、ウマ抗胸腺細胞グロブリンに代わってウサギ抗胸腺細胞グロブリン(rabbit antithymocyte globulin; rATG)が用いられている。本研究はAA患者に対するrATGの有効性と安全性を検討することを目的とする。

方法: 2009年5月から2013年12月までに当院でAAと診断し、rATGを用いて治療した17例の連続患者を解析した。1例は前治療としてシクロスポリン単剤による治療歴があったが、残り16例は初回治療であった。

結果: 年齢分布は8歳から81歳、中央値は68歳であった。重症度は非重症(non-severe AA; NSAA)5例、重症(severe AA; SAA)7例、最重症(very severe AA, VSAA)5例であった。発作性夜間血色素尿症(paroxysmal nocturnal hemoglobinuria; PNH)型血球は11例で陽性であった。rATGの投与量は2.5または3.75 mg/kg/dayであった。シクロスポリンが1例を除いて併用された。治療開始後6か月の時点で、14例の評価可能患者のうち10例(71%)に部分奏功(partial response; PR)を認め、最終的に2例が完全奏功(complete response; CR)を達成した。NSAAとSAA患者12例のうち9例(75%)がPRまたはCRの効果を示したが、6か月以上フォローした4例のVSAA患者のうち3例はPRの基準に到達しなかった。治療前後でPNH型血球の割合は変化しなかった。3例が死亡したが、いずれも75歳以上であった。死亡例のうち2例が心肺停止状態で救急搬送された。観察期間中央値は504日、2年生存率は79%であった。

結論: rATGは造血細胞移植非適応のNSAAおよびSAA患者に対して有効な治療法であると考えられる。一方、高齢者にrATGを用いる際は十分注意を払う必要がある。

キーワード: 再生不良性貧血、ウサギ抗胸腺細胞グロブリン、高齢者、PNH型血球