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Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a first course of intensive immunosuppressive therapy

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Summary. About 30% of patients with severe aplastic anaemia (SAA) unresponsive to one course of immunosuppressive (IS) therapy with antithymocyte or antilymphocyte globulin can achieve complete or partial remission after a second IS treatment. Among various second-line treatments, rabbit ATG (r-ATG) could represent a safe and effective alternative to horse ALG (h-ALG). In a multicentre study, 30 patients with SAA (17 males and 13 females, median age 21 years, range 2–67) not responding to a first course with h-ALG plus cyclosporin (CyA) and granulocyte colony stimulating factor (G-CSF), were given a second course using r-ATG (3·5 mg/kg/d for 5 d), CyA (5 mg/kg orally from day 1 to 180) and G-CSF (5 μg/kg subcutaneously from day 1 to 90). The median interval between first and second treatment was 151 d (range 58–361 d). No relevant side-effects were observed, but one patient died early during treatment because of sepsis. Overall response, defined as transfusion independence, was achieved in 23/30 (77%) patients after a median time of 95 d (range 14–377). Nine patients (30%) achieved complete remission (neutrophils $\geqslant 2{\cdot}0\times10^9$/l, haemoglobin $\geqslant$11 g/dl and platelets $\geqslant 100\times10^9$/l). The overall survival rate was 93% with a median follow-up of 914 d (range 121–2278). So far, no patient has relapsed. Female gender was significantly associated with a poorer likelihood to respond ($P = 0{\cdot}0006$). These data suggest that r-ATG is a safe and effective alternative to h-ALG for SAA patients unresponsive to first-line IS treatment.

Keywords: aplastic anaemia, immunosuppressive therapy, rabbit antithymocyte globulin, horse antilymphocyte globulin, cyclosporin.

Immunosuppressive (IS) therapy based on antilymphocyte (ALG) or antithymocyte globulin (ATG) and cyclosporine (CyA) greatly improved the response rate in patients with severe aplastic anaemia (SAA) not eligible for bone marrow transplant (Frickhofen *et al*, 1991). Furthermore, after the introduction of granulocyte colony stimulating factor (G-CSF), promising results were obtained also in patients with very low neutrophil counts ($<0{\cdot}2\times10^9$/l). In addition to the reduction of early deaths, partial or complete response, defined as trilineage haematological reconstitution and transfusion independence, was obtained in up to 80% of cases (Bacigalupo *et al*, 1995). Unfortunately, 10–20% of

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patients fail to respond to IS treatment and 30–35% relapse after an initially successful treatment (Schrezenmeier *et al*, 1993).

Some authors have reported consistent data on the efficacy of retreatment of relapsing or refractory patients with a second IS course. Doney *et al* (1984) reported response in 3/4 patients relapsing after a second course of ATG. Marsh *et al* (1987) reported a 22% response rate with rabbit ATG (r-ATG) in patients failing a previous course with horse ALG (h-ALG). Schrezenmeier *et al* (1993) reported that half of 74 relapsing patients responded to a second IS treatment. This series included 214 cases failing the first IS course, 67 of whom were retreated with r-ATG, but no separate analysis of these cases was provided. The Vanderbilt University group (Means *et al*, 1988; Stein *et al*, 1994) evaluated r-ALG as an alternative to h-ALG in 57 patients also receiving prednisone and/or androgens and/or cyclosporin: 8/20 retreated with h-ALG after r-ALG and 2/8 retreated with r-ALG after h-ALG responded. Moreover, in a recent single-centre study, 25 patients failing one course of h-ALG were treated with a second or more course of h-ALG (Tichelli *et al*, 1998). The response rate was 64% with a 55 ± 10% probability of survival at 10 years.

Because of its greater supply h-ALG is usually the first choice, but treating patients with a second course of ALG from the same source might increase the risk of allergic reactions. In fact, Tichelli *et al* (1998) showed an increased rate of acute reactions of immediate hypersensitivity type after retreatment with h-ALG (11% *v* 2%, $P = 0{\cdot}05$). Accordingly, a rationale for treating refractory or relapsing patients with ALG from a different animal could be the lower risk of potentially life-threatening allergic reactions.

Within the framework of Gruppo Italiano Trapianti di Midollo Osseo (GITMO), patients with SAA have been uniformly treated since 1991 with h-ALG, CyA and G-CSF (Bacigalupo *et al*, 1995). In this study we have analysed the clinical data and outcome of 30 patients failing one IS course of h-ALG, CyA and G-CSF who were treated again with a similar IS course substituting h-ALG with r-ATG.

MATERIALS AND METHODS

Patients. From 1 October 1991 a total of 100 patients with acquired SAA from 15 different Italian Centres were enrolled in a clinical trial assessing a schedule of h-ALG (15 mg/kg i.v., days 1–5), CyA (5 mg/kg, orally, days 1–180) and with G-CSF (5 μg/kg days 1–90). SAA was defined as hypoplastic bone marrow with <5% blasts, neutrophil count $\leq 0{\cdot}5 \times 10^9$/l, platelet count $\leq 20 \times 10^9$/l and anaemia requiring red blood cell support. For non-responders, bone marrow transplantation, if a suitable donor was available, or second-line therapy with r-ATG, CyA and G-CSF, was planned. 'Non-responders' were defined as those patients with pancytopenia requiring blood cell transfusion 120 d after the start of the first IS course (Bacigalupo *et al*, 1995).

Up to August 1997, 30 non-responders received a second IS course. Clinical details at the time of first treatment are summarized in Table I. At the time of second IS all patients were dependent on red blood cells and platelet transfusions. Relapsing cases were not included.

Table I. Clinical characteristics of patients retreated with rabbit ATG.

	Median	Range
Age (years)	18·5	2–67
Sex (male/female)	17/13	
Neutrophils ($\times 10^9$/l)	0·2	0–5
Platelets ($\times 10^9$/l)	5	1–33
Haemorrhages (yes/no)	14/16	
Infection (yes/no)	11/19	
Interval between diagnosis and first treatment (d)	27	4–457
Interval between first and second treatment (d)	151	58–361
Follow-up (d)	914	121–2278

Treatment. Rabbit ATG (Thymoglobulin; Merieux, Lyon, France), 3·5 mg/kg, diluted in isotonic saline, was infused i.v. over 6–8 h from day 1 to 5, according to the manufacturer's instructions; methyl prednisolone 2 mg/kg, from day 1 to 5 and 1 mg/kg from day 6 to 10 was administered i.v. and tapered within 30 d; CyA 5 mg/kg was given orally, from day 1 to 180 and slowly tapered thereafter. 24 patients received G-CSF (filgrastim) 5 μg/kg subcutaneously, from day 1 to 90.

Prophylactic broad-spectrum antibiotics and antimicotics were given orally.

Irradiated platelet concentrates and packed red blood cells were given for platelet $<15 \times 10^9$/l and Hb <8·5 g/dl.

Response. Patients were assessed for clinical response 180 d after the start of the second IS course. A period of transfusion independence of at least 1 month was required to define a case as a responder. Patients were classified as complete responders if they had a neutrophil count $\geq 2{\cdot}0 \times 10^9$/l, platelet count $\geq 100 \times 10^9$/l and a haemoglobin level 11 g/dl; partial responders if they had neutrophil count $>0{\cdot}5 \times 10^9$/l, platelet count $>20 \times 10^9$/l and a haemoglobin level >8 g/dl.

Statistical analysis. All the patients entered descriptive, univariate and multivariate analysis. Survival was calculated by the Kaplan-Meier method. Two patients who received bone marrow transplantation (BMT) were censored at that time.

RESULTS

Overall, the treatment was well tolerated; no anaphylactic reactions or severe side-effects were recorded. Seven patients (23%), all female, remained transfusion dependent with minimal improvement in their blood cell counts. Two (6·6%) of them died. The first, a 67-year-old woman died because of sepsis 30 d after r-ATG. The second, a 13-year-old girl, died of infection (neutrophil count $<1 \times 10^9$/l) 1 year after treatment. One patient underwent allogeneic bone marrow transplant (BMT) from HLA-matched unrelated donor 21 months after treatment. 23 patients (77%) became transfusion independent after a median time of 95 d (range 14–377). Nine patients (30%) had a complete response, after a median time of 61 d

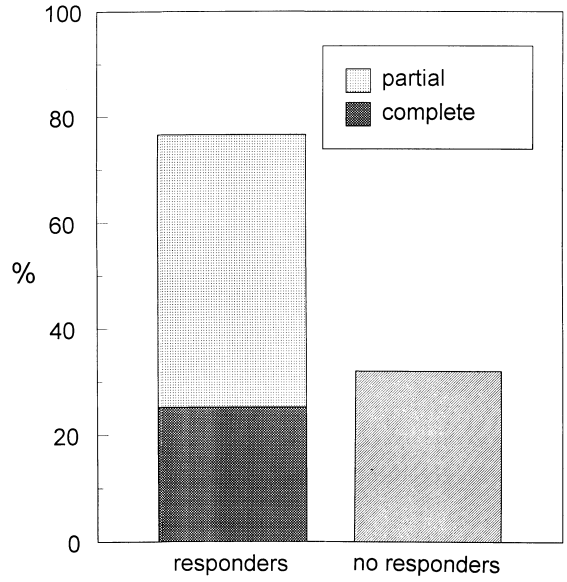

Fig 1. Response after rabbit ATG.

(range 14–196); 14 (47%) achieved partial remission after a median period of 150 d (range 23–377) (Fig 1). 10 patients were re-treated between 85 and 120 d (Table I) after the first IS course on a clinical basis by at the discretion of the attending physician. No difference in response rate (7/10 *v* 16/20) or in the median time to response (941 *v* 1055 d, *P* not significant) was observed between this group of patients and those treated 120 d after the first course. No difference was observed between partial and complete responders apart from time to response (150 *v* 61 d, respectively, $P = 0{\cdot}04$) (Fig 2). One partial responder received allogeneic BMT 18 months after the start of treatment. Of the six patients who did not receive G-CSF, two were non-responders, one partial and three complete responders.

At the last observation, 7/23 (30%) responders were not receiving any drug, after a mean period of 27 months (range 9–34) since the last transfusion. Most patients with partial (10/12) and complete (4/9) response continued cyclosporine with or without tailored dosage of G-CSF for a mean period of 23 months (range 0·7–69) since the last transfusion. Recovery of neutrophil and platelet count was significantly different in partial and complete responders (Table II).

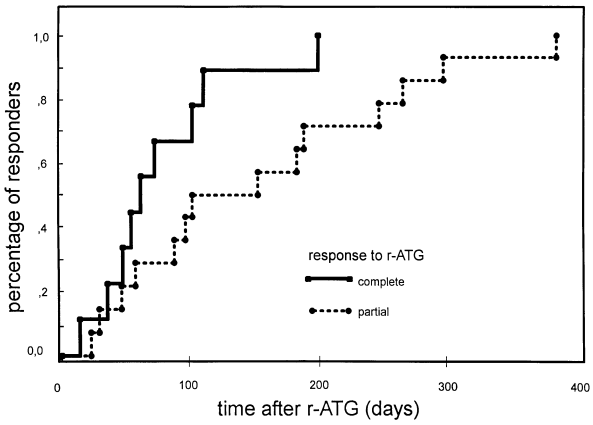

Fig 2. Time to response after rabbit ATG according to partial (14 cases) or complete (nine cases) response. The difference is statistically significant ($P = 0{\cdot}04$).

Table II. Haematological recovery in partial and complete responders (median and range values).

	Partial	Complete	*P*
Patients (*n*)	14	9	
Sex (male/female)	10/4	7/2	0·7
Haemoglobin (g/dl)	11·8 (9·7–15)	13 (11·5–15)	0·1
Neutrophils ($\times 10^9$/l)	1·3 (0·2–2·3)	2·1 (2–5)	0·0008
Platelets ($\times 10^9$/l)	58 (40–117)	140 (100–212)	0·002
Interval between first and second treatment (d)	167 (58–226)	126 (89–361)	0·3
Interval between second treatment and response (d)	150 (23–377)	61 (14–196)	0·04

All patients had a normal cytogenetic profile demonstrated at diagnosis or sometimes during the first 6 months of follow-up period and at the last observation. Three patients developed a clonal disease: two myelodysplasia (6·5%) and one monoclonal gammopathy of undefined significance (3%). No relapses have been observed to date.

The only response-influencing variable was sex; 7/13 women were unresponsive, whereas all males responded ($P = 0{\cdot}0006$, Fisher exact test).

The median follow-up for surviving patients is 914 d (range 121–2278). Actuarial survival is 93% (Fig 3): 100% for responders and 71% for not responders ($P = 0{\cdot}01$). Survival was not influenced by sex (100% and 85% for male and female respectively), age, interval between diagnosis and first treatment or between first and second treatment, complete or partial response.

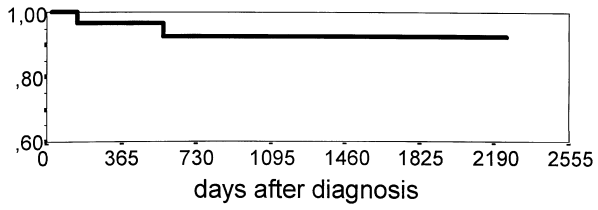

Fig 3. Overall survival after rabbit ATG in a cohort of 30 patients not responding to a first immunosuppressive course with horse ALG, cyclosporin and filgrastim.

DISCUSSION

We have shown in this study that SAA patients not responsive to first-line intensive IS treatment have a 77% chance of achieving remission and a 93% chance of survival

after r-ATG based second-line treatment. Median follow-up of surviving patients is 30 months. No anaphylaxis or major symptoms of allergic reaction were recorded.

Other reports have examined the effectiveness of a second course of ATG (Doney *et al*, 1984; Marsh *et al*, 1987; Means *et al*, 1988; Schrezenmeier *et al*, 1993, 1995; Stein *et al*, 1994; Tichelli *et al*, 1998). The present series, although limited in number, is unique for the following reasons: all patients received the same first-line therapy, consisting of h-ALG, CyA, G-CSF (Bacigalupo *et al*, 1995); all patients received r-ATG as second-line treatment in association with CyA with or without and G-CSF; all patients were treated and followed prospectively within the same co-operative group. Whether some of these patients would have responded in the absence of a second-line therapy seems unlikely. In fact, the interval between ALG and response in first- and second-line therapy was the same. In our series the probability of becoming transfusion independent was similar after the first course with h-ALG or after retreatment with r-ATG (70% *v* 77%, respectively). Furthermore, Tichelli *et al* (1998) have confirmed the efficacy of a second or subsequent course with h-ALG, reporting a 63% response rate, completely superimposable to that obtained after the first course (89/139, 64%). These data suggest that retreatment with ALG is effective and that r-ATG has at least the same efficacy when compared with h-ALG. In contrast to Tichelli *et al* (1998), who reported a significant higher rate of acute reactions (11% *v* 2%, $P = 0{\cdot}05$), no relevant immediate or late side-effects were observed after r-ATG.

Twenty-three patients achieved transfusion independence. Time to response was similar to that reported in first-line therapy with h-ALG (Bacigalupo *et al*, 1995). 50% of the patients responded within 95 d, but the range was wide and transfusion independence was achieved in 90% of the patients within 1 year after treatment. Our findings are similar to that reported by Tichelli *et al* (1998), who found 8/27 responding patients with haematological recovery after 6 months or more.

Slow responders were often partial responders; partial and complete responders differed in time to response and in haematological recovery (Table II). The different quality of haematological recovery may be due to the various pathogenic mechanisms involved in AA (Young & Maciejewski, 1997). This is in keeping with the residual abnormalities of haemopoiesis observed *in vitro* (i.e. proliferative capacity of haemopoietic progenitors) (Bacigalupo *et al*, 1993; Podestà *et al*, 1998; Piaggio *et al*, 1998).

At the last follow-up, only 33% of responders were off therapy, whereas most continued cyclosporine with or without tailored dosage of G-CSF. The long-lasting treatment may explain the absence of relapses. It is known that some patients may relapse after the discontinuation of therapy with CyA (Gluckman *et al*, 1992; A. Bacigalupo, personal communication) and that discontinuation of G-CSF induces a dramatic reduction of the neutrophil count.

Female gender appeared the only factor predicting failure, suggesting an hormonal influence. No differences were observed between males and females as to WBC, platelet count, age at the time of the first therapy, time to the first treatment, interval between treatments. A different response rate between males and females has been reported in a randomized study comparing the effectiveness of h-ALG with or without androgens (Bacigalupo *et al*, 1993). Responding females were 20% *v* 39% of males in the group treated with h-ALG alone, but the adjunct of androgen conferred a significant higher advantage to female gender (60% *v* 43%).

Evolution to a clonal haematological disorder is matter of concern. The incidence of myelodysplasia, acute leukaemia or solid neoplasm after one or more immunosuppressive courses varied between about 10% (De Planque *et al*, 1989; Socie *et al*, 1993) and 40%, including paroxysmal nocturnal haemoglobinuria (Tichelli *et al*, 1998). The degree of immunosuppression could play a role in clonal evolution. Tichelli *et al* (1998) have found a slight, but not significant, difference between patients exposed to one or more immunosuppressive courses (34% and 53% respectively, $P = 0{\cdot}2$), whereas Socie *et al* (1993) reported a higher risk (relative risk 2·26, $P = 0{\cdot}03$) after repeated courses. Although the number of patients was small, we observed a clonal disorder only in 10% of the cases after a second IS therapy with r-ATG, CyA and G-CSF. Furthermore, in our series, in keeping with data reported by De Planque *et al* (1989), the development of a clonal disorder did not influence survival. Actually, given the high incidence of myelodysplastic features in peripheral blood and bone marrow at diagnosis (Tichelli *et al*, 1992), it is sometimes difficult to distinguish between hypoplastic myelodysplastic syndrome and aplastic anaemia on morphological grounds. The presence of cytogenetic abnormalities strictly correlate with clonal evolution (Mikhailova *et al*, 1996) and cytogenetics combined with fluorescent *in situ* hybridization should be used to reveal non-random chromosomal abnormalities (La Starza *et al*, 1998).

In conclusion, although a control arm was not included, our data support the use of rabbit ATG as second-line treatment for patients failing to respond to IS treatment based on horse ALG. The high rate of responses in this negatively selected group of patients warrants a randomized prospective trial comparing rabbit ATG to horse ALG as a first choice. The excellent survival suggests that, at present, bone marrow transplant from a non-sibling donor should be postponed to third-line therapy.

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