Arch. Immunol. Ther. Exp. (2012) 60:99–106
DOI 10.1007/s00005-012-0164-3

REVIEW

Antibodies in the Treatment of Aplastic Anemia

David Gómez-Almaguer · Jose Carlos Jaime-Pérez · Guillermo J. Ruiz-Arguelles

Received: 1 July 2011 / Accepted: 28 November 2011 / Published online: 4 February 2012

Abstract Antibodies have been the cornerstone of treatment of acquired aplastic anemia for more than 25 years. Treatment with antithymocyte globulin (ATG) is considered pivotal and the addition of cyclosporine improves the overall response rate. This antibody is heterogeneous and horse ATG is apparently more effective than rabbit ATG. Several issues remain unsolved in relation to the combination of ATG and cyclosporine: cost, toxicity and late clonal disorders. In recent years, alternative immunosuppressive therapy has been proposed and new antibodies have emerged: porcine ATG, alemtuzumab, daclizumab, and rituximab. Experience with these antibodies is limited to a few studies with alemtuzumab being the most promising, but the results are interesting and provocative. More studies are needed to find the perfect antibody.

Keywords Aplastic anemia · Antibodies · Alemtuzumab · Antithymocyte globulin

Introduction

Aplastic anemia (AA) is the most conspicuous member of the group of disorders known as bone marrow failure syndromes, a defined group of alterations of hematopoiesis affecting one or more cell lineages. In adults AA is considered acquired and is almost always immune in origin (Kojima et al. 2011; Young 2000; Young and Maciejewski 1997), with exceptions being exposure to toxins, viral infections, drug-related, or iatrogenic.

That an immune mechanism was the cause of most adult AA cases became evident four decades ago, when it was shown that in vitro T cells from AA patients suppressed hematopoiesis (Ruiz-Argüelles et al. 1984); these inhibitory T cells were later identified as belonging to the $CD8^+$ subset (Zoumbos et al. 1985). Another piece of evidence appeared after it was shown that some AA patients, in whom grafting from an allogeneic bone marrow donor had failed, were able to recover endogenous hematopoiesis, suggesting that the immunosuppressive pre-transplant conditioning regimen was responsible for the cure of the disease (Thomas et al. 1972). With the introduction of molecular biology methods it was shown that the mechanisms causing damage include the T cell, direct stem cell lesion and the anti-proliferative effects of some of the resultant inhibitory cytokines, including interferon γ, tumor necrosis factor α, and transforming growth factor (Dufour et al. 2009).

Sometimes AA results from congenital or hereditary disorders; however, in most cases the etiology is unknown and considered to be idiopathic. In most idiopathic cases, autoimmune mechanisms are the cause of AA. This is evident because therapeutic responses to infusions of antithymocyte globulins (ATG) and other immunosuppressive agents are consistently observed (Champlin et al. 1983). The incidence of acquired AA in North America and Europe is close to 2/1,000,000 per year, being two or three times higher in East Asia, with a bimodal age distribution at 10–25 and >60 years (Marsh et al. 2003).

D. Gómez-Almaguer (✉) · J. C. Jaime-Pérez
Hematology Service, Hospital Universitario, UANL,
Paris 3029 Col. Cumbres, 64610 Monterrey, N.L., Mexico
e-mail: dr_gomez@infosel.net.mx

G. J. Ruiz-Arguelles
Centro de Hematología y Medicina Interna de Puebla,
Puebla, Mexico

Current Mechanism and Future Targets in AA

Hematopoietic failure in acquired AA is mediated by activated cytotoxic T cells in blood and marrow and by the biological products released upon their activation (Dufour et al. 2009; Zoumbos et al. 1985). These cytokines are both, suppressive and destructive, leading to cell death in the stem cell compartment, most probably through Fas-mediated apoptosis (Maciejewski et al. 1995). Identification of an in vivo dominant clonal immune response in patients with AA thus reveals the underlying antigen-driven immune process. Although the target of the aberrant immune response is the hematopoietic stem cell, the triggering antigen(s) remains unknown and constitutes the basis of AA research. The combination of cell phenotypic, molecular biology, and functional analyses to study the effector arm of immunity will help to establish a molecular signature of disease activity and precisely define an immune pathophysiology in AA (Risitano et al. 2004), conceivably leading to the design of specifically tailored therapies in individual patients.

The classic autoimmunity theory states that the target tissue is normal with altered self-recognition mechanisms being responsible for the self-attack. An alternative theory for AA postulates that the autoimmune attack is instead aimed at eliminating transforming neoplastic cells, based on the presence of abnormal apoptosis-resistant hematopoietic clones, which could be the primary target of AA, with normal stem cells destroyed as innocent bystanders (Nissen and Schubert 2002). Under these circumstances immunosuppressive treatment (IST) for AA would be considered a two-edged sword, with dose-intensity accordingly adapted to the patient's immune competence.

Clonal T cells that have the hematopoietic stem cell as their attack goal have been identified through T-cell receptor analysis. Others have identified auto-antibodies to different proteins in the serum of AA patients; however, definitive proof that these auto-antibodies are indeed essential for the development of AA and that suppressing their synthesis significantly contributes to alleviate the disease is lacking. In this respect, a recent study identified auto-antibodies to a novel auto-antigen, heterogeneous nuclear ribonucleoprotein K, in 85 of 273 (31%) AA patients (Qi et al. 2010). Alternatively, it has been proposed that these auto-antibodies only reflect a more general background of altered autoimmunity in AA patients (Risitano 2011). Possible immune mechanisms implicated in the pathophysiology of acquired AA are summarized in Table 1.

From the previous information, it can be understood that manipulation of the AA patients' immune system, aimed at either regulating or stopping the self-directed hematopoietic autoimmune attack is the best rationale in treatment approach. The most radical method in this respect is represented by allogeneic stem cell transplantation from a HLA-identical donor, which is possible in less than 30% of the cases. For almost three decades the standard approach for 75% or so of individuals without a stem cell donor has been immunosuppressive therapy, based essentially on a combination of ATG plus cyclosporine A (Marsh et al. 2003). There are, however, numerous unsolved issues for this immunosuppressive regimen, among others the relapse rate and high cost, which leads to a numerous group of patients in developing countries with no access to this regimen (Shereck et al. 2011).

Table 1 Immune mechanisms involved in the pathogenesis of acquired AA

$CD8^+$ T cells attack the stem cell (Zoumbos et al. 1985)
Anti-proliferative cytokines and Fas-mediated apoptosis (Maciejewski et al. 1995)
Innocent bystander stem cells (Nissen and Schubert 2002)
Auto-antibodies to hnRNP K protein (Qi et al. 2010)

hnRNP heterogeneous nuclear ribonucleoprotein

Antithymocyte Globulin

Stemming from evidences of immune-mediated suppression of hematopoiesis in most patients with acquired AA, IST have been designed for individuals who, for different reasons, can not be allografted (Delgado-Lamas et al. 1989; Gómez-Almaguer et al. 2006; Ruiz-Argüelles 2009; Ruiz-Argüelles and Díaz-Jouanen 1979; Young and Maciejewski 1997).

The first experience that formally proved that IST with ATG may lead to marrow function recovery was reported by Speck et al. (1977). They showed that ATG alone was as effective as ATG followed by an infusion of allogeneic bone marrow in terms of hematological improvement and survival, and that it was possibly better than allogeneic hematopoietic stem cell transplantation (Speck et al. 1977). The efficacy of ATG was subsequently confirmed by other groups and, based on the results of a prospective randomized trial by Champlin et al. (1983), IST became the standard of care for AA patients in who transplant was not an option (Champlin et al. 1983; Frickhofen et al. 1991). Response rates using ATG ranged between 30 and 70% in larger series, which also pointed out the risk of late treatment failure due to disease relapse (Di Bona et al. 1999). The subsequent aim for investigators was to increase the response rate and maintain responses for longer, preventing subsequent relapses. A number of immunosuppressive agents were initially combined with ATG: corticosteroids, androgens and cyclosporin A (CyA) (Di Bona et al. 1999).

Only CyA, a calcineurin inhibitor that impairs interleukin (IL)-2-dependent T-cell activation and differentiation, has proven to be effective in increasing the response rate, as initially demonstrated by Frickhofen et al. (1991), Risitano et al. (2010); accordingly ATG plus CyA has been the most employed IST for severe AA (SAA) patients in the past two decades.

Unfortunately, mainly because of cost, access to such treatment still represents a problem in developing countries. In addition to industrial production of ATG, limited batches of horse ATG have been prepared domestically (Ruiz-Argüelles et al. 1986) and employed successfully as treatment (Delgado-Lamas et al. 1989). Overall, the trials which have analyzed the effectiveness of ATG in SAA show that 57–77% of patients respond, with an overall survival of 55–93% at 3–11 years (Champlin et al. 1983; Risitano et al. 2010; Ruiz-Argüelles and Gómez-Rangel 2003).

ATG is a heterologous anti-serum obtained by injecting human lymphocytes in different animals. Various ATG preparations exist, which differ in both the stimulating antigens (peripheral lymphocytes, thymocytes or even T-cell lines), and/or the host animal (horse, rabbit or pig) (Bing et al. 2010; Passweg and Tichelli 2009; Risitano et al. 2010) (Table 2). In the last two decades, physicians have employed several commercially available ATG preparations without distinction. Data from large randomized clinical trials refer to polyclonal equine ATGs obtained from horse (h); North-American investigators have used hATG (25–40 mg/kg/day for 4–5 days) (ATGAM®), which is different from the hATG preparation used in Europe and Japan (15 mg/kg/day for 5 days) (Lymphoglobulin®). Both preparations can be considered equivalent as standard IST for AA; however, Lymphoglobulin is no longer available, and in some European countries no hATG preparation is available (Bacigalupo et al. 1995; Kojima et al. 2011). Alternative polyclonal ATGs are the leporine ones, obtained from rabbits (r). Two rATGs are currently available (Thymoglobulin® by Genzyme and ATG® by Fresenius), but to date the clinical results with these agents are less robust due to the lack of large randomized trials. Thymoglobulin has been used in AA patients, and both retrospective data and prospective series have demonstrated substantial efficacy, possibly equal to that of hATG. In most cases, rATG is used as second-line IST to prevent side effects due to possible sensitization to horse proteins, resulting in response rates up to 68% (Di Bona et al. 1999; Zheng et al. 2006). As front-line therapy, experience with Thymoglobulin is limited. Recently, the Spanish group (Vallejo et al. 2009) reported a large study showing a 45% response rate. The European Group for Blood and Marrow Transplantation (EBMT) is retrieving its retrospective registry to assess the actual response rate to Thymoglobulin in comparison to historical data using Lymphoglobulin. A recent similar study from Brazil suggests that rATG may not be as effective as hATG (Atta et al. 2010). The US National Institutes of Health is currently running a large randomized study comparing head-to-head hATG (ATGAM®) with rATG (Thymoglobulin®), both arms with the addition of CyA as first-line treatment for AA patients; recruitment is almost complete, and the results, until now, show that rATG is markedly inferior to hATG with the 6-month response being 35 versus 69%, respectively ($p = 0.0017$) (Scheinberg et al. 2010a). It is important to note that in patients treated with ATG, the risk of myelodysplastic syndrome (MDS) evolution is around 21% at 10 years (Maciejewski and Risitano 2005).

Given that some concerns exist on the dose-equivalence between Thymoglobulin and Lymphoglobulin, the EBMT is running a pilot study to investigate whether 2.5 mg/kg/day of Thymoglobulin (for 5 days) is equivalent to the most utilized dose (3.75 mg/kg/day). Unlike Thymoglobulin, robust clinical data on the use of ATG-Fresenius are lacking; only anecdotal experiences are available, and the only prospective randomized series from China showed clinical results significantly inferior to hATG (Zheng et al. 2006). Based on these results at the moment ATG-Fresenius cannot be considered a standard IST for patients with AA. Porcine ATG has also been employed successfully in

Table 2 Salient features of some of the ATGs employed in the treatment of patients with severe AA

Origin	Brand	Company	Dose	References
Equine	ATGAM	Upjohn	40 mg/kg/day × 4 days	Rosenfeld et al. (2003)
Equine	Lymphoglobulin	Genzyme	15 mg/kg/day × 5 days	Frickhofen et al. (2003)
Equine	Domestic	AMEH	2 mg/kg/day × 5 days	Ruiz-Argüelles et al. (1986), Delgado-Lamas et al. (1989)
Leporine	Thymoglobulin	Genzyme	2.5–3.75 mg/kg/day × 7 days	Di Bona et al. (1999), Scheinberg et al. (2006)
Leporine	ATG	Fresenius	2–5 mg/kg/day × 5 days	Zheng et al. (2006)
Porcine	Domestic	Wohan Institute	25 mg/kg/day × 5 days	Bing et al. (2010)

AMEH Agrupación Mexicana para el Estudio de la Hematología

the treatment of SAA in China (Bing et al. 2010), but comparative studies with hATG are not available; it has been claimed that porcine ATG is substantially cheaper than both equine and leporine.

Other Antibodies

Alternative immunosuppressive regimens have the intention of improving current results, without the toxicity, cost, and long-term complications related to the use of ATG. Therefore, the use of monoclonal antibodies and other biological agents has been studied for treatment of AA patients. We have limited information in this setting; three antibodies have been employed in small series of patients or anecdotal cases: alemtuzumab, daclizumab, and rituximab (Castiglione et al. 2006; Gómez-Almaguer et al. 2010a; Hansen and Lauritzen 2005; Kim et al. 2009; Risitano et al. 2010; Sloand et al. 2010a, b; Takamatsu et al. 2011).

Alemtuzumab

Alemtuzumab is a monoclonal IgG1 antibody directed at the CD52 antigen expressed on the membrane of several cells including B and T lymphocytes. It induces lymphoablation through antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity.

This antibody has been used in the treatment of several autoimmune hematological diseases such as hemolytic anemia, pure red cell aplasia, immune thrombocytopenic purpura, and as part of the conditioning regimen for HSCT in AA patients (Gómez-Almaguer et al. 2008, 2010b; Grupta et al. 2005; Selleri et al. 2011; Willis et al. 2001). Alemtuzumab spares progenitor or stem cells, but is clearly cytotoxic for lymphocytes, resulting in a prolonged decrease in these cells, specially CD4 and CD8 lymphocyte population. Since the drug induces a profound immunosuppression without causing damage to stem cells, its use in patients with AA that have a hyperactive immune system is reasonable (Selleri et al. 2011).

Few reports that include only small series of AA patients treated with alemtuzumab (Table 3) have been published. Kim et al. (2009) studied 19 patients, 17 with AA, one with hypoplastic type of MDS and one with pure red cell aplasia. Median age was 48 years, including 14 patients with a severe and very severe form of disease; 13 were free of any specific prior treatment when they received alemtuzumab (Kim et al. 2009). They employed two different doses with the best response obtained using 60 mg (50%). The antibody was administered by intravenous infusion, 10 mg on day 1, 20 mg on day 2, and 30 mg on day 3. Cyclosporine 1.5 mg/kg twice a day was added and maintained for 6 months. Five patients received 90 mg of alemtuzumab with no response. Median time to initial response was 2.07 months, toxicity was acceptable and median overall survival at 2 years was 81.6%.

Risitano et al. (2010), studied 35 patients, but only 6 patients suffering from AA were untreated and prospectively analyzed. Thirteen patients with AA were studied after failing first-line IST. The other 16 patients had single lineage marrow failure. Alemtuzumab was administered subcutaneously to the AA patients, 11 received 103 mg, 5 received 73 mg, 2 received 100 mg, and in 1 patient the dose was not registered in the table. Cyclosporine was also added as well as prophylactic antibiotics. The response rate was 58%, but among the six AA patients receiving the antibody as first-line therapy, there was one partial response and three complete responses (66%). Toxicity was, as in the study by Kim et al. (2009), limited and acceptable (Risitano et al. 2010).

We prospectively studied 14 AA patients, all of who had not received any kind of disease specific therapy. Median age was 23 years (range 6–78). We included five children.

Alemtuzumab, 10 mg, was administered subcutaneously daily for 5 days. In addition, cyclosporine, 2 mg/kg twice a

Table 3 Alemtuzumab as first-line therapy in patients with AA

AuDisease	Patients	Median age	Dose (mg)	Results	References
SAA	6		60	2 CR, 4 NR	
TDAA	2		60	1 CR, 1 PR	
SAA	2	48	90	2 NR	Kim et al. (2009)
TDAA	1		90	1 NR	
VSAA	2		90	2 NR	
SAA	4		103	2 CR, 1 PR, 1 NR	Risitano et al. (2010)
SAA	2		73	1 CR, 1 NR	
SAA	11		50	1 CR, 5 PR, 5 NR	
VSAA	2	23	50	1 CR, 1 NR	Gómez-Almaguer et al. (2010a)
NSAA	1		50	1 PR	

SAA severe aplastic anemia, *TDAA* transfusion-dependent non-severe aplastic anemia, *VSAA* very severe aplastic anemia, *NSAA* non-severe aplastic anemia, *CR* complete response, *PR* partial response, *NR* no response

day, was given for 3 months and then tapered during the fourth month. Prophylactic antibiotics were also used. Eight of the 14 patients responded, but only 2 complete responses were obtained; 6 patients achieved a partial response and median time to response was 2.5 months. The overall response was 57.1% and overall survival was 71% at 38 months. The drug was very well tolerated, no serious toxicity was observed and no patient, until now, has acquired a late clonal disorder (Gómez-Almaguer et al. 2010a).

Investigators from the US National Institutes of Health, used alemtuzumab as monotherapy for severe AA patients. Surprisingly, they found that the antibody was effective in AA patients with relapsed or refractory disease, but the results in treatment-naive patients were disappointing, since only 3 patients from a total of 16 obtained a response, and moreover, there were 3 early deaths. No cyclosporine was used in this trial and alemtuzumab was given at 10 mg/day for 10 days (Scheinberg et al. 2010b). In this setting, it is important to note that the use of cyclosporine is considered essential in the treatment of AA patients, in fact it has been proposed that cyclosporine A tapering is best done over many years to reduce the risk of relapse (Passweg and Tichelli 2009; Saracco et al. 2008).

Sloand et al. (2010a) conducted a pilot study in patients suffering from MDS who were likely to respond to immunosuppression. Alemtuzumab 10 mg/day intravenously for 10 days was administered. Seventeen of 22 intermediate-one patients (77%), and 4 of 7 intermediate-two patients (57%) responded to treatment with a median response time of 3 months. Furthermore, 56% of evaluable patients at 12 months had normal blood counts, and 78% were transfusion independent (Sloand et al. 2010a). From these data we can conclude that the number of untreated AA patients treated with the combination of alemtuzumab and cyclosporine and prospectively studied is only 33 (not including the myelodysplasia patients). The response is about 50–60% and appears after 2 months. Cyclosporine is needed, as well as prophylactic antibiotics, to sustain the response and avoid common viral and other infections. The drug is well tolerated and toxicity is expected to be not serious. However, how can we compare these results with those obtained using ATG and cyclosporine? The answer is clear, by means of a prospective and randomized study comparing ATG plus cyclosporine versus alemtuzumab plus cyclosporine, a very complicated study to perform. Meanwhile, alemtuzumab is a good alternative for patients who, for any reason, are unsuitable to receive treatment with stem cell transplantation or conventional immunosuppression.

Daclizumab

This monoclonal antibody was genetically engineered and humanized. It is a IgG 1 antibody that specifically recognizes an epitope of the IL-2) receptor associated to T lymphocyte activation (CD25). Daclizumab has been used, with some success, in the treatment of multiple sclerosis, to avoid rejection in kidney transplantation, in patients with acute graft versus host disease, and in immune-mediated uveitis. One group of investigators used this antibody in patients with moderate AA, 42% responded, but of the 28 patients who were transfusion dependent at the time of treatment only seven achieved transfusion independence (Maciejewski et al. 2003). Recently long-term results of the use of daclizumab have been reported, the 5-year survival is over 90%, but there is no difference between responders and non-responders (Sloand et al. 2010b).

Rituximab

Rituximab is a chimeric antibody very active against B lymphocytes. It is directed against the CD20 antigen found on these lymphocytes. Nowadays this antibody is not only used for treatment of lymphomas, but also as therapy for many patients with autoimmune diseases, including immune-mediated hemolytic anemia and thrombocytopenia.

In AA patients, affected by a predominant T cell-mediated immune disorder, the use of rituximab is not expected to be effective. However, it has been suggested that rituximab has an indirect effect on T-cell lymphocytes, since B cells are antigen presenting cells and also have the capability to provide co-stimulatory signals that lead to T-cell activation (Linton et al. 2000; Shlomchik et al. 2001; Stasi 2010), furthermore, there is a cumulative evidence of circulating autoantibodies in patients with AA. On the other hand, the unexpected response of three patients with hairy cell leukemia, a B-cell disorder, to immunosuppressive therapy with ATG plus cyclosporine, also suggests some role of B cell-mediated suppression of hematopoietic stem cells in patients with AA (Fuijiwara et al. 2009; Sugimori et al. 2003).

To the best of our knowledge, there are only three case reports published regarding the effectiveness of rituximab in AA. The first patient was a 73-year-old woman with no specific therapy for AA before the use of rituximab. She achieved a nearly complete recovery after therapy with the antibody. Another patient was a 68-year-old woman with chronic lymphocytic leukemia who developed bone marrow failure after receiving one cycle of fludarabine and cyclophosphamide. She had a partial recovery with the use of rituximab. Finally a 1-year-old boy with AA, resistant to ATG and cycloporine, was treated with rituximab and achieved complete remission. He was doing well with a normal complete blood count 4 years after the administration of rituximab.

Other Observations

As far as economic aspects of these treatments are concerned, it is interesting that a single course of hATG (ATGAM®) or rATG (Thymoglobulin®) costs between 10 and 15,000 USD in Mexico, whereas the cost of 50 mg of alemtuzumab is around 1,300 USD, making this alternative very attractive for the majority of our uninsured patients. In our setting, we and others have been able to reduce the cost of HSCT to 15–20,000 USD, mainly using non-myeloablative, fludarabine-based conditioning regimens (Gómez-Almaguer et al. 2006; Jaime-Pérez et al. 2005; Ruiz-Argüelles and Gómez-Almaguer 2008). These data mean that conventional IST is almost as expensive as HSCT in Mexico as a therapeutic option for patients with AA. Therefore, it is important to predict the response to immunosuppressive therapy and survival in severe AA. It has been proposed that younger age, absolute reticulocyte count $>$ or $=25 \times 10^9$/l, and absolute lymphocyte count $>$ or $=1 \times 10^9$/l, are highly predictive of response at 6 months, with the 5-year survival of patients meeting these criteria reaching 92% (Scheinberg et al. 2009b). Therapeutic decisions, especially in developing countries, should not overlook these predictive and economical aspects.

There have been several attempts to improve the response to conventional IST by adding other drugs like sirolimus and mycophenolate, but the results so far have been disappointing (Scheinberg et al. 2006, 2009a). Modified androgens have long been used as therapy in some countries, and recently we reported an overall response rate of 45.9% and a 5-year survival of almost 60% employing danazol, a modified non-virilizing androgen (Jaime-Perez et al. 2011). The addition of this drug to conventional IST improved the response in Japan (Kamio et al. 2011; Shereck et al. 2011), but after withdrawal of danazol many patients relapsed, suggesting the need to explore the possible role of danazol in this subgroup of AA patients as a maintenance agent. Finally, we have used the combination of low doses of alemtuzumab and rituximab with success in patients with autoimmune cytopenias (Gómez-Almaguer et al. 2010b) and we are going to use this combination in a pilot trial for patients with AA.

Conclusions

There is no doubt that the combination of cyclosporine and hATG remains the sole standard treatment of AA patients, especially when the option of a stem cell transplant is not possible for any reason. However, there is still trouble in this setting. ATG preparations are heterogeneous. At least in Europe and Asia, rabbit ATG is currently in use, but the antibody is dosed differently and apparently is not as effective as horse ATG. Available data from randomized studies confirm that the polyclonal ATG obtained from horse should be considered the first choice for immunosuppression-based AA treatment. On the other hand, alemtuzumab is emerging as a new possible immunosuppressive alternative, since ATG is associated with high cost, toxicity, late relapses and clonal disorders. Rituximab and other antibodies against B cells should not be forgotten and could be considered in future clinical trials. There is still room for improvement of current immunosuppressive regimens.

References

Atta EH, Dias DS, Marra VL et al (2010) Comparison between horse and rabbit antithymocyte globulin as first-line treatment for patients with severe aplastic anemia: a single center retrospective study. Ann Hematol 89:851–859

Bacigalupo A, Broccia G, Corda G et al (1995) Antilymphocyte globulin, cyclosporin and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA Working Party. Blood 85:1348–1353

Bing H, Siyi Y, Weg Z et al (2010) The use of anti-human T lymphocyte porcine immunoglobulin and cyclosporine A to treat patients with acquired severe aplastic anemia. Acta Haematol 124:245–250

Castiglione MG, Scatena P, Pandolfo C et al (2006) Rituximab therapy of severe aplastic anemia induced by fludarabine and cyclophosphamide in a patient affected by B-cell chronic lymphocytic leukemia. Leuk Lymphoma 47:1985–1986

Champlin R, Ho W, Gale RP (1983) Antithymocyte globulin treatment in patients with aplastic anemia: a prospective randomized trial. N Engl J Med 308:113–118

Delgado-Lamas JL, López-Karpovitch X, Marín-López A et al (1989) Low-doses of high-potency antithymocyte globulin (ATG) in severe aplastic anemia: experience with the Mexican ATG. Acta Haematol 81:70–74

Di Bona E, Rodeghiero F, Bruno B et al (1999) 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. Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Br J Haematol 107:330–334

Dufour C, Ferretti E, Bagnasco F et al (2009) Changes in cytokine profile pre and post immunosuppression in acquired aplastic anemia. Haematologica 94:1743–1747

Frickhofen N, Kaltwasser JP, Schrezenmeier H et al (1991) Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German Aplastic Anemia Study Group. N Engl J Med 324:1297–1304

Frickhofen N, Heimpel H, Kaltwasser JP et al (2003) Antithymocyte globulin with or without cyclosporine A: 11-year follow-up of a randomised trial comparing treatments of aplastic anemia. Blood 101:1236–1242

Fuijiwara S, Miyake H, Nosaka K (2009) Hairy cell leukemia responsive to anti-thymocyte globulin used as immunosuppressive therapy for aplastic anemia. Int J Hematol 90:471–475

Gómez-Almaguer D, Vela-Ojeda J, Jaime-Pérez JC et al (2006) Allografting in patients with severe aplastic anemia using peripheral blood stem cells and a fludarabine-based conditoning regimen: the Mexican Experience. Am J Hematol 81:157–161

Gómez-Almaguer D, Ruiz-Argüelles GJ, Tarín-Arzaga LC et al (2008) Alemtuzumab for the treatment of steroid-refractory acute graft-versus-host disease. Biol Blood Marrow Transplant 14:10–15

Gómez-Almaguer D, Jaime-Perez JC, Garza-Rodriguez V et al (2010a) Subcutaneous alemtuzumab plus cyclosporine for the treatment of aplastic anemia. Ann Hematol 89:299–303

Gómez-Almaguer D, Solano-Genesta M, Tarín-Arzaga L et al (2010b) Low dose rituximab and alemtuzumab combination therapy for patients with steroid-refractory autoinmune cytopenias. Blood 116:4783–4785

Grupta V, Ball SE, Sage D et al (2005) Marrow transplants from matched unrelated donors for aplastic anemia using alemtuzumab, fludarabine and cyclophosphamide based conditioning. Bone Marrow Transplant 35:467–471

Hansen PB, Lauritzen AM (2005) Aplastic anemia successfully treated with rituximab. Am J Hematol 80:292–294

Jaime-Perez JC, Colunga-Pedraza PR, Gomez-Ramirez CD et al (2011) Danazol as first-line therapy for aplastic anemia. Ann Hematol 90:523–527

Jaime-Pérez JC, Ruiz-Argüelles GJ, Gómez-Almaguer D (2005) Haematopoietic stem cell transplantation to treat aplastic anemia. Expert Opin Biol Ther 5:617–626

Kamio T, Ito E, Ohara A et al (2011) Relapse of aplastic anemia in children after immunossuppresive therapy: a report from the Japan Childhood Aplastic Anemia Study Group. Haematologica 96:814–819

Kim H, Min YJ, Baeck JH et al (2009) A pilot dose-escalating study of alemtuzumab plus cyclosporine for patients with bone marrow failure syndrome. Leuk Res 33:222–231

Kojima S, Nakao S, Young N et al (2011) The Third Consensus Conference on the treatment of aplastic anemia. Int J Hematol 93:832–837

Linton PJ, Harbertson J, Bradley LM (2000) A critical role for B cells in the development of memory CD4 cells. J Immunol 165:5558–5565

Maciejewski JP, Risitano AM (2005) Aplastic anemia: management of adult patients. Hematology Am Soc Hematol Educ Program 2005:110–117

Maciejewski JP, Selleri C, Sato T et al (1995) Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. Br J Haematol 91:245–252

Maciejewski JP, Sloand EM, Nuñez O et al (2003) Recombinant humanized anti-IL-2 receptor antibody (daclizumab) produces responses in patients with moderate aplastic anemia. Blood 102:3584–3586

Marsh JC, Ball SE, Darbyshire P et al (2003) Guidelines for the diagnosis and management of acquired aplastic anaemia. Br J Haematol 123:782–801

Nissen C, Schubert J (2002) Seeing the good and bad in aplastic anemia: is autoimmunity in AA dysregulated or antineoplastic? Hematol J 3:169–175

Passweg J, Tichelli A (2009) Immunosuppressive treatment for aplastic anemia: are we hitting the ceiling? Haematologica 94: 310–312

Qi Z, Takamatsu H, Espinoza JL et al (2010) Autoantibodies specific to hnRNP K: a new diagnostic marker for immune pathophysiology in aplastic anemia. Ann Hematol 89:1255–1263

Risitano AM (2011) Immunosuppresive therapies in the management of immune-mediated marrow failures in adults: where we stand and where we are going. Br J Haematol 152:127–140

Risitano AM, Maciejewski J, Green S et al (2004) In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing. Lancet 364:355–364

Risitano AM, Selleri C, Serio B et al (2010) Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anemia and single-lineage bone marrow failure: a pilot study and a survey from the EBMT WPSSAA. Br J Haematol 148:791–796

Rosenfeld S, Follmann D, Nunez O et al (2003) Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematopoietic response and long term outcome. JAMA 289:1130–1135

Ruiz-Argüelles GJ (2009) Hipoplasias y displasias medulares. In: Ruiz-Argüelles GJ (ed) Fundamentos de Hematología, 4th edn. Editorial Médica Panamericana, México, pp 117–127

Ruiz-Argüelles GJ, Díaz-Jouanen E (1979) Existen formas autoinmunes de aplasia medular? Rev Invest Clin Méx 32:195–196

Ruiz-Argüelles GJ, Gómez-Almaguer D (2008) Making allogeneic bone marrow transplantation available to patients in developing countries: the Mexican Experience. Open Hematol J 2:30–36

Ruiz-Argüelles GJ, Gómez-Rangel D (2003) Long term results of the immunosuppressive treatment of patients with severe, acquired aplastic anemia: a single institution study. Acta Haematol 110:184–187

Ruiz-Argüelles GJ, Katzmann JA, Greipp PR et al (1984) Lymphocyte subsets in patients with aplastic anemia. Am J Hematol 16:267–275

Ruiz-Argüelles A, Salvador Ruiz-González JD, Miguel Presno-Bernal J (1986) Preparation and characteristics of an equine anti-human thymocyte gamma globulin produced in Mexico (in Spanish). Rev Invest Clin 38:273–278

Saracco P, Quarelli P, Iori AP et al (2008) Cyclosporin A response and dependence in children with acquired aplastic anemia: a multicentre retrospective study with long-term observation follow-up. Br J Haematol 140:197–205

Scheinberg P, Nuñez O, Wu C et al (2006) Treatment of severe aplastic anaemia with combined immunosuppression: anthytimocyte globulin, cyclosporine and mycophenolate mofetil. Br J Haematol 133:606–611

Scheinberg P, Wu CO, Nuñez O et al (2009a) Treatment of severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, with and without sirolimus, a prospective randomized study. Haematologica 94:348–354

Scheinberg P, Wu CO, Nuñez O et al (2009b) Predicting response to immunosuppressive therapy and survival in severe aplastic anaemia. Br J Haematol 144:206–216

Scheinberg P, Wu CO, Scheinberg P et al (2010a) A randomized trial of horse versus rabbit antithymocyte globulin in severe acquired aplastic anemia. Blood 116:a4

Scheinberg P, Wu CO, Scheinberg P et al (2010b) Alemtuzumab (Campath) monotherapy for severe aplastic anemia. Blood 116:a1167

Selleri C, Serio B, Risitano AM (2011) Novel immunosuppressive strategies for bone marrow failure syndromes: a focus on Alemtuzumab. Mini Rev Med Chem 11:536–543

Shereck EB, Deyell RJ, Kurre P (2011) Costs and consequences of immunosuppressive therapy in children with aplastic anemia. Haematologica 96:793–795

Shlomchik MJ, Craft JE, Mamula MJ (2001) From T to B and back again: positive feedback in systemic autoimmune disease. Nat Rev Immunol 1:147–153

Sloand EM, Olnes MJ, Shenoy A et al (2010a) Alemtuzumab treatment of intermediate-1 myelodysplasia patients is associated with sustained improvement in blood counts and cytogenetic remission. J Clin Oncol 28:5166–5173

Sloand EM, Olnes MJ, Weinstein B et al (2010b) Long-term follow-up of patients with moderate aplastic anemia and pure red cell aplasia treated with daclizumab. Haematologica 95:382–387

Speck B, Gluckman E, Haak HL et al (1977) Treatment of aplastic anaemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions. Lancet 2:1145–1148

Stasi R (2010) Rituximab in autoimmune hematologic diseases: not just a matter of B cells. Semin Hematol 47:170–179

Sugimori C, Kaito K, Nakao S (2003) Persistent remission after immunosuppressive therapy of hairy cell leukemia mimicking aplastic anemia: two case reports. Int J Hematol 77:391–394

Takamatsu H, Yagasaki H, Takahashi Y et al (2011) Aplastic anemia successfully treated with rituximab: the possible role of aplastic anemia-associated autoantibodies as a marker for response. Eur J Haematol 86:541–545

Thomas ED, Storb R, Fefer A et al (1972) Aplastic anemia treated by marrow transplantation. Lancet 1:284–289

Vallejo C, Montesinos P, Rosell A et al (2009) Comparison between lymphoglobulin- and thymoglobulin-based immunosuppressive therapy as first-line treatment for patients with aplastic anemia. Blood 114:a3194

Willis F, Marsh JC, Bevan DH et al (2001) The effect of treatment with Campath-1H in patients with autoimmune cytopenias. Br J Haematol 114:891–898

Young NS (2000) Acquired aplastic anemia. In: Schechter GP, Berliner N, Telen MJ (eds) Hematology 2000. The American Society of Hematology Education Program Book, Washington, pp 18–38

Young NS, Maciejewski JP (1997) The pathophysiology of acquired aplastic anemia. N Engl J Med 336:1365–1372

Zheng Y, Liu Y, Chu Y et al (2006) Immunosuppressive therapy for acquired severe aplastic anemia (SAA): a prospective comparison of four different regimens. Exp Hematol 34:826–831

Zoumbos NC, Gascón P, Djeu JY et al (1985) Circulating activated suppressor T lymphocytes in Aplastic anemia. N Engl J Med 312:257–265