

Aplastic anemia: immunosuppressive therapy in 2010

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Abstract

Acquired aplastic anemia (AA) is the typical bone marrow failure syndrome characterized by an empty bone marrow; an immune-mediated pathophysiology has been demonstrated by experimental works as well as by clinical observations. Immunosuppressive therapy (IST) is a key treatment strategy for aplastic anemia; since 20 years the standard IST for AA patients has been anti-thymocyte globuline (ATG) plus cyclosporine A (CyA), which results in response rates ranging between 50% and 70%, and even higher overall survival. However, primary and secondary failures after IST remain frequent, and to date all attempts aiming to overcome this problem have been unfruitful. Here we review the state of the art of IST for AA in 2010, focusing on possible strategies to improve current treatments. We also discuss very recent data which question the equality of different ATG preparations, leading to a possible reconsideration of the current standards of care for AA patients.

Introduction

Aplastic anemia (AA) is the most typical example of bone marrow failure, characterized by an empty or fatty bone marrow leading to the subsequent pancytopenia.¹ Recently it has been reported that in some adults AA may be due to inherited abnormalities.² However, idiopathic AA is usually considered an immune-mediated disease.³ According to the most accepted view, self-reactive T cells cause a damage of the hematopoietic stem cells (and possibly of committed progenitors) through a cell-cell interaction (via Fas/Fas-L, granzyme, perforine)⁴ and the production of inhibitory cytokines such as IFN- γ , TNF- α and TGF- β .⁵⁻⁷ The role of T cells was confirmed by the identification, *in vivo*, of oligoclonal T cells,⁸⁻¹⁰ and by the demonstration of their pathogenic role either *in vitro* or *in vivo*.^{10,11} All these findings make the immune system the therapeutic target in AA patients; immunosuppressive (IS) regimens have been largely developed in the past

years, exploiting agents that affect distinct steps of the immune response.

Immunosuppression for aplastic anemia

The standard immunosuppression: anti-thymocyte globuline plus cyclosporine A

Initial observations showed that some AA patients failing donor engraftment following allogeneic stem cell transplantation rescued autologous hematopoiesis¹² and that in other patients treatment with anti-lymphocyte globuline (ALG) resulted beneficial.^{13,14} In fact, the efficacy of immunosuppressive treatment (IST) by ALG was confirmed in a prospective, placebo-controlled, randomized trial, in 1983.¹⁵ In order to improve the response rate and reduce the risk of subsequent relapse, several immunosuppressive agents have been associated to anti-thymocyte globuline (ATG) or ALG (such as corticosteroids,¹⁶ androgens)^{17,18} but cyclosporine A (CyA) only resulted in an increased response rate,¹⁹ with an improved long-term failure-free survival.²⁰ Since the early '90s, ATG + CyA was considered the standard IST for AA patients, with an expected 50-60% probability of response and 60% overall survival at one year.²¹⁻²³

The most recent studies have shown improved overall survival (above 80% at 1 year), regardless of the initial response to IST;²⁴⁻²⁶ likely due to a better supportive care and salvage treatment (mainly SCT). However, treatment-failure remains a major problem after first-line IST. In fact, about one third of AA patients do not respond to their initial IST; in addition, within responders patients, half of them require long-term IS maintenance treatment by CyA to sustain the response. In fact, recent studies showed that CyA-dependency ranges between 25 and 50% of patients and the patients who require long-term CyA treatment present the higher risk to relapse (about 30-50% of responders).²²⁻²⁴ Furthermore, the development of clinical paroxysmal nocturnal hemoglobinuria is seen in about 10% of AA patients after IST;²⁷ clonal evolution to myelodysplastic syndromes (MDS) or acute leukemias (AML) accounts for about 10-15% of treatment-failures,^{24,28} and solid tumors account for an additional 10%.²⁹ Thus, a substantial fraction of AA patients cannot be considered cured by IST, and understanding the underlying causes is necessary to develop salvage strategies.³⁰ While secondary failures suggest a flare-up of the underlying immune process, the causes accounting for primary failures (which occur in one third of patients) may include: i) non-immune pathophysiology (e.g., due to misdiagnosis of hypoplastic MDS, or to inherited forms associated to mutation in telomerase complex genes);³¹ ii) an insufficient delivered IS (in fact, some refractory patients may respond to further IST);³²⁻³⁴

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iii) a third explanation is the exhaustion of the hematopoietic stem cells, which would hamper any hematological recovery regardless the control of the pathogenic immune-attack. This latter hypothesis seems supported by the recent data showing that baseline telomere length is the most powerful predictor of long-term survival in AA patients receiving IST.³⁵ In fact, shorter telomeres were associated with increased relapse rate and clonal evolution (including monosomy 7), suggesting that they are a reliable marker for functional hematopoietic stem cell damage (possibly linked to the replicative stress of residual cells). If confirmed, these data will provide an informative tool to identify AA patients who may benefit from an early transplant strategy rather than IST.

Improving standard ATG-based immunosuppression: additional or alternative IS agents

To improve the results obtained with the standard ATG + CyA, several investigators tried to deliver an intensified IS by adding a third IS agent, possibly with a distinct (hopefully synergistic) mechanism of action. However, this strategy did not result in a substantial benefit. The purine synthesis inhibitor mycophenolate mofetil (MMF) was tested in a prospective study conducted at NIH, but did not result in either increased response (62% at 6 months) or decreased relapse (37%, despite maintenance therapy with MMF) in comparison to historical data.³⁶ The mammalian target of rapamycin (mTOR) inhibitor rapamycin/sirolimus (RAPA)³⁷ was also tested in a randomized trial conducted at NIH; the addition of RAPA to the standard ATG + CyA resulted in a response rate of 51%, which was comparable to that of the control arm (ATG + CyA, 62%), with similar relapse and survival rates.³⁸

Some investigators developed different strategies of IST, with the aim of retaining (or possibly increasing) a marked IS activity, ideally with a better toxicity profile. They used lymphocyte depleting agents other than ATG, such as cyclophosphamide (CTX) or alemtuzumab. High-dose CTX (50 mg/kg intravenously on 4 consecutive days) was mainly tested at the Johns Hopkins University; the initial results were excellent, with a response rate of about 70% (even if the time-to-response appeared delayed in comparison to that expected with ATG).³⁹ This single-center experience continues to show interesting results, with the most recent follow up reporting 44 naïve AA patients showing response rate, overall survival and event free survival of 88%, 71% (the majority complete) and 58%, respectively.⁴⁰ However, most investigators do not consider CTX as a feasible treatment option for AA patients, based on the results of the randomized study versus ATG + CyA conducted at NIH. This study was early stopped due to increased fatal infectious complications in the experimental arm (CTX + CyA), related to the prolonged neutropenia resulting from CTX myelotoxicity;⁴¹ in addition, the latest follow up did not confirm Johns Hopkins' data suggesting that CTX may reduce the risk of MDS/AML development.⁴²

Another candidate agent for inducing lymphocyte depletion in AA patients is the anti-CD52 monoclonal antibody alemtuzumab, which specifically kills CD52-bearing cells via both antibody-dependent cellular cytotoxicity and complement-mediated lysis. We have recently reported, in collaboration with the EBMT Working Party for Severe Aplastic Anemia (WPSAA), that an alemtuzumab-based IS regimen (also including low-dose CyA) was feasible, safe and effective for the treatment of AA patients.⁴³ Alemtuzumab was given subcutaneously with negligible injection-related side effects, and the low rate of infectious complications ruled out most safety concerns; preliminary efficacy data suggested response rates not below standard IS regimen (58%), with easy re-treatment in case of relapse. These data confirms observations from smaller series.^{44,45} However, recent data from NIH seem only partially confirm these positive results;⁴⁶ in fact, alemtuzumab resulted in a 56% response rate in relapsed AA (n=23) and 36% in refractory AA (n=25). However, quite surprisingly, as front line treatment alemtuzumab resulted in a response rate as low as 19% (on 16 patients only); it has to be remarked that in the NIH experience alemtuzumab was used as single agent, without CyA.

Other IS agents may be also hypothesized for the treatment of AA; in the past years, the anti-CD25 monoclonal antibody daclizumab was used for moderate forms of AA, with a response rate of about 40%.^{47,48} Given the proven

role of cytokines in suppressing the hematopoiesis in AA,⁷ some cytokine inhibitors have been hypothesized for the treatment of AA, especially the already available TNF- α inhibitors etanercept (a TNF-receptor/Ig fusion protein),⁴⁹ infliximab (a chimeric anti-TNF- α mAb) and adalimumab (a fully humanized anti-TNF- α mAb). One could also hypothesize that in the future additional IS agents will be investigated in AA, such as alefacept,⁵⁰ efalizumab,⁵¹ anti-IFN agents⁵² or even immunomodulation by mesenchymal stem cells.⁵³

Current immunosuppression: the matter of different ATG preparations

ATG is a heterologous anti-serum obtained by injecting human lymphocytes in animals; various ATG preparations exist, which differ in stimulating antigens (peripheral lymphocytes, thymocytes or even T cell lines), and/or in the host animal (either horse or rabbit). Thus, even if comprehensive descriptions of the composition of each anti-serum are limited, they are obviously different;⁵⁴⁻⁵⁶ in addition, at least in the past, inter-lot variation due to manufacturing processes cannot be excluded.⁵⁷ The majority of available data coming from large randomized clinical trials refer to polyclonal ATGs obtained from horse (h), which have to be considered the gold standard for AA treatment. Of note, US and Japanese investigators utilized hATG (ATGAM®, Upjohn; 40 mg/kg/day for 4 days),^{24,25} which is different from the hATG preparation used in Europe (Lymphoglobuline®, Genzyme; 15 mg/kg/day for 5 days).^{20,26} Even if a formal head-to-head comparison has been never conducted, both h-ATG preparations resulted in response rates ranging between 50% and 70%;^{20,24-26} thus, they are considered equivalent as standard IST for AA. However, since 2008 Lymphoglobuline is no longer available in Europe, and physicians were forced to utilize other ATG preparations. Alternative polyclonal ATGs may be obtained from rabbits (r); two rATGs are currently available (Thymoglobuline®, Genzyme; ATG-Fresenius®) but to date the clinical results with these agents are less robust for the lack of large randomized trials. Thymoglobuline has been utilized in AA patients, and both retrospective data and prospective series have demonstrated a substantial efficacy. In most cases, rATG was used as second-line IST (after initial hATG) to prevent side effects due to possible sensitization to horse proteins, resulting in response rates up to 68% (in relapsed patients).^{33,34} As a front-line therapy, the experience with Thymoglobuline is quite limited; the only prospective trial is currently ongoing at NIH, where investigators are comparing head-to-head h-ATG (ATGAM) and r-ATG (Thymoglobuline), both arms with CyA, as first line treatment for AA patients (NCT00260689). The

recruitment is now closed (60 patients per arm), and preliminary data were just presented at the 2010 ASH meeting;⁵⁸ very surprisingly, r-ATG was markedly inferior to h-ATG in terms of response rate (33% vs 62% and 35% vs 68% at 3 and 6 months, respectively). Of note, lymphocyte depletion after r-ATG was markedly longer-lasting in comparison to h-ATG, raising the question that lymphocyte depletion may be not sufficient to achieve hematological remission in AA patients. Based on this data, one could hypothesize that additional immune or non-immune mechanisms may be involved in the pathophysiology of AA, and that h-ATG may target them more efficiently than r-ATG. For instance, regardless the antibodies resulting in T cell depletion, h-ATG might contain antibodies targeting immune cytokines involved in the inhibition or in the damage of hematopoietic stem cells. The NIH data were in agreement with a retrospective study from Brazil,⁵⁹ which showed a 34.5% response rate with Thymoglobuline in comparison to the 59.5% achieved with Lymphoglobuline (patients were 42 and 29, respectively). However, other retrospective experiences are in contrast with the results of this randomized study: investigators at the Cleveland Clinic treated 22 naïve AA patient with Thymoglobuline, showing a response rate of 50% and 54% at 6 and 12 months, respectively; these results were comparable to the historical control of 67 AA patients who have received initial treatment with ATGAM (59% response rate at 6 and 12 months).⁶⁰ A similar retrospective study was conducted by the Spanish group,⁶¹ which showed that 75 AA patients receiving front-line treatment with Thymoglobuline (2.5 mg/kg for 5 days) had a response rate of 45%, which was comparable to the 49% of the 35 patients who have received Lymphoglobuline. Additional studies are currently ongoing to investigate the efficacy of Thymoglobuline: the EBMT is retrieving its retrospective registry to assess the actual response rate. In addition, the EBMT is running a pilot study (NCT00471848) to investigate whether 2.5 mg/kg/day of Thymoglobuline (for 5 days) may be equivalent to the most utilized dose (3.75 mg/kg/day). Indeed, based on these data, at the moment h-ATG + CyA seems the safest treatment for AA patients; Thymoglobuline, which have in any case a substantial efficacy, should be recommended only if h-ATGs are not available, or within prospective trials. Finally, ATG-Fresenius should not be used as IST for AA patients, given that its use is limited to anecdotic and disappointing experiences.⁶²

Conclusions

To date, ATG + CyA remains the standard IST for AA patients. All the attempts to improve

the results obtained with this regimen have been unfruitful. Unexpectedly, recent observations have confounded rather than clarified our knowledge of IST in AA; in fact, the dogma that different ATG preparations may be equally effective has been unexpectedly debunked. The fact that hematological response does not correlate with lymphocyte depletion also suggest that h-ATG may work through unknown mechanisms of actions, even other than immune, leading to a possible dispute of AA pathophysiology itself. Even if these data have to be confirmed, at the moment h-ATG seems the best standard of care for AA patients; this represents a urgent challenge for those Countries (i.e., Europe) where h-ATG is no longer available. At the moment, it is not clear whether novel IS agents or strategies may be useful to improve the results of current IST; the design of large, co-operative prospective studies seems the only way to unravel the open issues in IS for the treatment of AA.

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