Aplastic anemia: immnosuppressive 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. Recently it has been reported that in some adults AA may be due to inherited abnormalities. However, idiopathic AA is usually considered an immune-mediated disease. 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, perforin) and the production of inhibitory cytokines such as IFN-γ, TNF-α and TGF-β. The role of T cells was confirmed by the identification, in vivo, of oligoclonal T cells, and by the demonstration of their pathogenic role either in vitro or in vivo. 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 hematopoiesis and that in other patients treatment with anti-lymphocyte globuline (ALG) resulted beneficial. In fact, the efficacy of immunosuppressive treatment (IST) by ALG was confirmed in a prospective, placebo-controlled, randomized trial, in 1983. 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, androgens) but cyclosporine A (CyA) only resulted in an increased response rate, with an improved long-term failure-free survival. 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.

The most recent studies have shown improved overall survival (above 80% at 1 year), regardless of the initial response to IST, 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). Furthermore, the development of clinical paroxysmal nocturnal hemoglobinuria is seen in about 10% of AA patients after IST; clonal evolution to myelodysplastic syndromes (MDS) or acute leukemias (AML) accounts for about 10-15% of treatment-failures and solid tumors account for an additional 10%. Thus, a substantial fraction of AA patients cannot be considered cured by IST, and understanding the underlying causes is necessary to develop salvage strategies. 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 muta-tion in telomerase complex genes); ii) an insufficient delivered IS (in fact, some refractory patients may respond to further IST); 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. 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. The mammalian target of rapamycin (mTOR) inhibitor rapamycin/sirolimus (RAPA) 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.
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, 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 patients, based on the results of the randomized study with ATG + CyA seems the safest 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 not be 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 Thymoglobulin in comparison to the 59.5% achieved with Lymphoglobuline (patients were 42 and 29, respectively).

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.54,55 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).30,54 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

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 suggests that h-ATG may work through unknown mechanisms of action, 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.

References

1. Young NS. Acquired aplastic anemia. In: Young NS, ed. Bone marrow failure syndromes. Philadelphia: WB Saunders. 2000;1-46.
2. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood 2006;108:2509-19.
3. Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. N Engl J Med 1997;336:1365-72.
4. Maciejewski JP, Selleri C, Sato T, et al. Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. Br J Haematol 1995;91:245-52.
5. Zoumbos NC, Gascon P, Djeu JY, Young NS. Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. Proc Natl Acad Sci U S A 1985;82:188-92.
6. Slaand E, Kim S, Maciejewski JP, Tisdale J, et al. Intracellular interferon-gamma in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anaemia. Br J Haematol 1995;91:245-52.
7. Dufour C, Ferretti E, Bagnasco F, et al. Marrow Failure Study Group of the AIEOP. Changes in cytokine profile pre- and post-immunosuppression in acquired aplastic anemia. Haematologica 2009;94:1743-7.
8. Zeng W, Nakao S, Takamatsu H, et al. Characterization of T-cell repertoire of the bone marrow in immune-mediated aplastic anemia: evidence for the involvement of antigen-driven T-cell response in cyclosporine-dependent aplastic anemia. Blood 1999;93:3008-16.
9. Risitano AM, Kook H, Zeng W, et al. Oligoclonal and polyclonal CD4 and CD8 lymphocytes in aplastic anemia and paroxysmal nocturnal hemoglobinuria measured by V beta CD3R3 spectratyping and flow cytometry. Blood 2002;100:178-83.
10. Risitano AM, Maciejewski JP, Green S, et al. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenic T-cell clones by TCR beta-CD3R3 sequencing. Lancet 2004;364:355-64.
11. Nakao S, Takami A, Takamatsu H, et al. Isolation of a T-cell clone showing HLA-DRB1*0405-restricted cytotoxicity for hematopoietic cells in a patient with aplastic anemia. Blood 1997;89:3691-9.
12. Mathé G, Schwarzenberg L. Treatment of bone marrow aplasia by mismatched bone marrow transplantation after conditioning with antilymphocyte globulin—long-term results. Transplant Proc 1976;8:595-602.
13. Speck B, Gluckman E, Haak HL, van Rood JJ. Treatment of aplastic anaemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions. Lancet 1977;2:1145-8.
14. Speck B, Gratwohl A, Nissen C, et al. Treatment of severe aplastic anemia with antilymphocyte globulin or bone-marrow transplantation. Br Med J (Clin Res Ed) 1981;282:860-3.
15. Champlin R, Ho W, Gale RP. Antithymocyte globulin treatment in patients with aplastic anemia: a prospective randomized trial. N Engl J Med 1983;308:113-8.
16. Doney K, Pepe M, Storb R, et al. Immunosuppressive therapy of aplastic anemia: results of a prospective, randomized trial of antithymocyte globulin (ATG), methylprednisolone, and oxymetholone to ATG, very high-dose methylprednisolone, and oxymetholone. Blood 1992;79:2566-71.
17. Champlin RE, Ho WG, Feig SA, et al. Do androgens enhance the response to antithymocyte globulin in patients with aplastic anemia? A prospective randomized trial. Blood 1985;66:184-8.
18. Bacigalupo A, Chaple M, Hows J, et al. Treatment of aplastic anaemia (AA) with antithymocyte globulin (ATG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. Br J Haematol 1993;83:145-51.
19. Frichhofen N, Kaltwasser JP, Schrezenmeier H, et al. Treatment of aplastic anemia with antithymocyte globulin and methylprednisolone with or without cyclosporine. The German Aplastic Anemia Study Group. N Engl J Med 1991;324:1297-304.
cyte globulin for severe aplastic anaemia. Br J Haematol 1998;100:393-400.
33. Di Bona E, Rodighiero F, Bruno B, et al. Rabbit antithymocyte globulin (r-ATG) plus cyclophosphamide and granulocyte colony stimulating factor is an effective treatment for aplastic anemia patients unresponsive to a first course of intensive immunosuppressive therapy. Gruppo Italiano Trapianto di Midollo Osseo (GITMO). Br J Haematol 1999;107:330-4.
34. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit antithymocyte globulin and ciclosporin for patients with relapsed or refractory severe aplastic anemia. Br J Haematol 2006;133:622-7.
35. Scheinberg P, Cooper JN, Sloand EM, et al. Association of telomere length of peripheral blood leukocytes with hematopoietic relapse, malignant transformation, and survival in severe aplastic anemia. The Journal of the American Medical Association, 2010;304:1358-64.
36. Scheinberg P, Nunez O, Wu C, Young NS. Treatment of severe aplastic anemia with combined immunosuppression: antithymocyte globulin, ciclosporin and mycophenolate mofetil. Br J Haematol 2006;133:606-11.
37. Sehgal SN. Rapamune® (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. Clin Biochem 1998;31:335-40.
38. Scheinberg P, Wu CO, Nunez O, et al. Treatment of severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, with or without sirolimus: a prospective randomized study. Haematologica 2009;94:348-54.
39. Brodsky RA, Sensenbrenner LL, Jones RJ. Complete remission in severe aplastic anaemia after high-dose cyclophosphamide without bone marrow transplantation. Blood 1996;87:491-4.
40. Brodsky RA, Chen AR, Dorr D, et al. High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up. Blood 2010;115:2136-41.
41. Tisdale JF, Dunn DE, Geller N, et al. High-dose cyclophosphamide in severe aplastic anemia: a randomised trial. Lancet 2000;356:1554-9.
42. Tisdale JF, Maciejewski JP, Nunez O, et al. Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial. Blood 2002;100:4668-70.
43. Risitano AM, Selleri C, Serio B, et al. Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA. Br J Haematol 2010;148:791-6.
44. Gómez-Almaguer D, Jaime-Pérez JC, Garza-Rodríguez V, et al. Subcutaneous alemtuzumab plus cyclosporine for the treatment of aplastic anemia. Ann Hematol 2010;89:299-303.
45. Kim H, Min YJ, Baek JH, et al. A pilot dose-escalating study of alemtuzumab plus cyclosporine for patients with bone marrow failure syndrome. Leuk Res 2009;33:222-31.
46. Scheinberg P, Wu CO, Scheinberg P, et al. Alemtuzumab (Campath) monotherapy for severe aplastic anemia. Blood 2010;116:1167a.
47. Maciejewski JP, Sloand EM, Nunez O, et al. Recombinant humanized anti-IL-2 receptor antibody (daclizumab) produces responses in patients with moderate aplastic anaemia. Blood 2003;102:3584-6.
48. Sloand EM, Olnes MJ, Weinstein B, et al. Long-term follow-up of patients with moderate aplastic anemia and pure red cell aplasia treated with daclizumab. Haematologica 2010;95:382-7.
49. Fouillard L, Benabdoum M, Bories D, et al. Engraftment of allogeneic mesenchymal stem cells in the bone marrow of a patient with severe idiopathic aplastic anemia improves stroma. Leukemia 2003;17:474-6.
50. Smith AG, O’Reilly RJ, Hansen JA, Martin PJ. Specific antibody-blocking activities in antilymphocyte globulin as correlates of efficacy for the treatment of aplastic anemia. Blood 1983;66:721-3.
51. Kawano Y, Nissen C, Gratwohl A, Speck B. Immunostimulatory effects of different antilymphocyte globulin preparations: a possible clue to their clinical effect. Br J Haematol 1988;68:115-9.
52. Bourdage JS, Hamlin DM. Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry. Transplantation 1995;59:1194-200.
53. Raefsky EL, Gascon P, Gratwohl A, et al. Biological and immunological characterization of ATG and ALG. Blood 1986;68:712-9.
54. Scheinberg P, Wu CO, Scheinberg P, et al. A randomized trial of horse versus rabbit antithymocyte globulin in severe acquired aplastic anemia. Blood 2010;116: LBA-4.
55. Atta EH, Dias DS, Marra VL, De Azevedo AM. Comparison between horse and rabbit antithymocyte globulin as first-line treatment for patients with severe aplastic anemia: a single-center retrospective study. Ann Hematol 2010;89:851-9.
56. Afsah M, Shaik M, Sugimoto Y, et al. Efficacy of rabbit anti-thymocyte globulin (ATG) compared to horse ATG in severe aplastic anemia. Blood 2010;116:2236a.
57. Vallejo C, Montesinos P, Rosell A, et al. Rabbit antithymocyte globulin for severe aplastic anaemia. Br J Haematol 1998;100:393-400.