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APLASTIC ANEMIA

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Abstract

Purpose of review—Most acquired aplastic anemia (AA) is the result of immune-mediated destruction of hematopoietic stem cells causing pancytopenia and an empty bone marrow, which can be successfully treated with either immunosuppressive therapy (IST) or hematopoietic stem-cell transplantation (HSCT).

Recent findings—In AA, oligoclonally expanded cytotoxic T-cells induce apoptosis of hematopoietic progenitors. T-bet, a transcription factor that binds to the interferon- γ promoter region, is up-regulated in AA T-cells. Regulatory T-cells are significantly reduced in patients' peripheral blood and in an AA murine model, infusion of regulatory T-cells ameliorates disease progression. In a minority of cases, loss-of-function mutations in telomerase complex genes may underlie disease development. Long term survival, once strongly linked to response to immunosuppressive therapy, can now be achieved even among non-responders due to significant advances in supportive care and better salvage treatments.

Summary—Evidence has accumulated in the recent years further corroborating an immune-mediated process underlying AA pathogenesis. HSCT from a matched sibling donor is preferred for children and young adults with severe AA, and IST is employed when HSCT is not feasible due to age, lack of a histocompatible sibling, co-morbidities, or by patient choice.

Keywords

aplastic anemia; anti-thymocyte globulin; bone marrow failure; stem cell transplantation; cyclosporine; pancytopenia

Introduction

In its severe form, aplastic anemia (AA) is a life-threatening bone marrow failure disorder which, if untreated, is associated with very high mortality. Hematopoietic stem cell transplantation (HSCT) offers an opportunity for cure, but most patients are not suitable candidates for this procedure due to advanced age, comorbidities, or lack of a histocompatible donor. For these patients, comparable long term survival is attainable with immunosuppressive treatment (IST) with anti-thymocyte globulin (ATG) and cyclosporine (CsA). Although several etiopathogenic triggers have been proposed in AA, the majority of cases are idiopathic, with a small percentage of cases occurring after an episode of seronegative hepatitis.

Epidemiology and Etiology

Acquired AA is a rare disease; almost half of cases occur during the first three decades of life. The incidence in Western countries is two cases per million per year and about 2-3-fold higher in Asia [1–3]. Benzene and pesticides, while epidemiologically associated, account for a small etiologic fraction. In rural Thailand, associated exposures to non-bottled water, as well as to certain animals, to animal fertilizer, and also to pesticides suggest an infectious etiology [2]. The rarity of acquired AA most probably is accounted for by a combination of infrequent exposure events, a diversity of host genetic predisposing factors, and individual differences in the immune response.

During the last century, AA was attributed to an idiosyncratic reaction to drug or chemical exposure. The association of medical drug use to AA is of great importance, as it is devastating to patients and physicians and presents serious legal consequences and problems in pharmaceutical drug development [4]. However, the study of idiosyncratic drug reactions, by definition extremely rare, is difficult and the only clear predisposition to abnormal drug metabolism underlying susceptibility is one study of a single individual exposed to carbamazepine published over 20 years ago [5]. Overrepresentation of drug metabolizing glutathione-S-transferase gene deletions have been observed in some series [6,7] but no reasonable mechanism has been developed for chloramphenicol.

Pregnancy and eosinophilic fasciitis are linked to AA. Five to ten percent of cases of AA follow an episode of seronegative hepatitis [8], but despite intensive efforts, an infectious agent has not been identified.

Pathophysiology

In most cases, AA behaves as an immune-mediated disease. An immune response dominated by oligoclonal expanded cytotoxic T-cells targets hematopoietic stem and progenitor cells, inducing their death via apoptosis and hematopoietic failure.

T-cell-mediated destruction of the bone marrow

Recovery of autologous hematopoiesis in patients who failed to engraft after stem cell transplant and responsiveness to immunosuppressive therapies are the major clinical evidences supporting an immune pathophysiology underlying acquired AA. Although a nonimmune pathophysiology has been inferred from a failure to respond to immunosuppression, refractoriness to therapy is also consistent with very severe stem cell depletion, a “spent” immune response, or immunological mechanisms resistant to current therapies.

Removal of lymphocytes from aplastic bone marrows improves colony numbers in tissue culture, and their addition to normal marrow inhibited hematopoiesis in vitro [9]. The effector cells within the lymphocyte subset are activated cytotoxic T cells bearing a Th1 profile, expressing and secreting interferon- γ [10]. T-bet, a transcription factor that binds to the interferon- γ promoter region and is critical for Th1 polarization, is up-regulated in T-cells of patients with AA [11]. Specific CD8+CD28– cell clones are expanded in AA peripheral blood, as manifest by skewed usage of the V β repertoire; and oligoclones recognize and induce apoptosis of autologous myeloid cells [12]. Regulatory T cells, which control and suppress auto-reactive T cells, are decreased at presentation in almost all patients with AA [13]. In a mouse model of immune-mediated marrow failure, addition of T regulatory cells abrogated pancytopenia induced by the infusion of lymph node cells [14].

Why T-cells are activated in AA is unclear. HLA-DR2 is over-represented among patients, suggesting a role for antigen recognition, and its presence is predictive of a better response to cyclosporine [15,16]. Polymorphisms in cytokine genes, associated with an increased immune response, also are more prevalent, such as for tumor necrosis factor- α (*TNF2*) promoter at -308 [17], interferon- γ [18], and interleukin 6 genes [19]. These alterations in nucleotide sequence and in gene regulation suggest a genetic basis for aberrant T cell activation in bone marrow failure.

Hematopoiesis

Immune attack leads to marrow failure. The targets, mainly CD34+ cells, are very few or absent in the aplastic bone marrow, and minimal numbers of colonies derive from committed progenitors in semisolid media, all reflecting the severe reduction in hematopoietic progenitor cells that defines the disease. The reduced number and function of the marrow is secondary to cell destruction, and apoptosis is prevalent among the few remaining elements [20–22]. Microarray analysis of the remaining CD34+ cells shows a transcriptome shifted towards apoptosis, cell death, and immune regulation [23].

A minority of AA cases may share pathophysiologic basis with inherited marrow failure syndromes. One peculiar feature of white blood cells in AA is short telomeres, observed in approximately a third of cases [24,25]. Although initially blamed on excessive stem cell turnover, telomere shortening in some cases of acquired AA and in dyskeratosis congenita, a constitutional marrow failure syndrome is due to mutations in components of the telomerase complex, causing low telomerase activity, progressive telomere erosion, and a deficient proliferative capacity of hematopoietic stem cells [26–29]. Family members who share the mutation, despite normal or near normal blood counts, have hypocellular marrows, reduced CD34+ cell counts and poor hematopoietic colony formation, increased hematopoietic growth factor levels, and of course short telomeres; some affected relatives may present with pulmonary fibrosis or liver cirrhosis in isolation. A few adult AA patients also have heterozygous mutations in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene (Shwachman-Diamond syndrome occurs when patient carries biallelic mutations) [30].

Clonal Evolution

AA may coexist or evolve to clonal disorders, as paroxysmal nocturnal hemoglobinuria (PNH), myelodysplasia (MDS), or acute myeloid leukemia (AML). The mechanisms linking immune-mediated and pre-malignant or malignant pathophysiologies are not well elucidated in marrow failure or in other human autoimmune diseases that predispose to cancer.

PNH

About 40–50% of patients with acquired AA have expanded populations of PNH cells, easily detected by flow cytometry due to the absence of glycosylphosphatidylinositol-linked membrane proteins, the result of somatic *PIG-A* gene mutations arising in hematopoietic stem cells [31]. Most clones are small and do not lead to clinical manifestations of hemolysis or thrombosis, but classic PNH can evolve to marrow failure (the AA/PNH syndrome) and all PNH patients show evidence of underlying hematopoietic deficiency. The global absence of large number of cell surface proteins in PNH has been hypothesized to allow escape and survival of a pre-existing mutant clone.

MDS

Aneuploidy develops in a minority of patients treated with immunosuppression over time, usually monosomy 7 and trisomy 8 [32]. AA patients who develop trisomy 8 usually respond to IST [33]. T-cell oligoclonal populations appear to recognize the aneuploid cells and specifically WT1

as an antigen, but target cells are not killed due to their expression of anti-apoptotic genes [34].

Monosomy 7 is the most frequent cytogenetic abnormality in evolving AA; it confers a poor prognosis: with typical associated refractory cytopenia or AML [35]. Monosomy 7 has been linked to exogenous use of G-CSF in AA [36,37] and laboratory studies suggest that aneuploid clones expand in an abnormal cytokine milieu rich in G-CSF due to the presence of a short G-CSF receptor isoform, which signals proliferation but not differentiation [38]. The presence of even small monosomy 7 clones in the bone marrow, as detected by FISH (but not by routine cytogenetics), is a poor prognostic indicator for response to IST [39].

Immunosuppressive therapy

Horse anti-thymocyte globulin (ATGAM (R); h-ATG) is the only drug approved by the Food and Drug Administration for the treatment of AA. While it is generally believed that h-ATG administration leads to depletion of immune competent cells, its exact mechanism of action remains unclear [40]. H-ATG preparations contain a variety of antibodies recognizing human T-cell epitopes, many directed against activated T-cells or activation antigens [41,42]. Although the decline in circulating levels of lymphocytes is transient, the number of activated T-cells is decreased for more prolonged periods of time; this effect is also reflected in decreased IFN- γ and possibly TNF production after h-ATG [10]. In contrast to ATG, CsA has a more selective inhibitory effect on T lymphocytes, suppressing early cellular response to antigenic and regulatory stimuli. By blocking expression of nuclear regulatory proteins, it leads to reduced T cell proliferation and activation with diminished release of cytokines such as interleukin-2 and interferon- γ . The combination of h-ATG and CsA is current standard therapy in severe AA (SAA) [43,44]. The benefits (and limitations) of this regimen as initial therapy have been quantitated in systematic studies in the US, Japan and Europe: overall response is achieved in about 2/3 of patients; the cumulative incidence of relapse among responders is approximately 20–30% and clonal evolution occurs in about 10–15% of cases [45–48]. The majority of responses to IST are not complete, notwithstanding, hematologic response almost always equates to cessation of transfusion, and multiple studies have shown a strong correlation between hematologic response and long term survival [45,49]. Pediatric population studies in general report a higher response rate of about 70–80% with long term survival of 80–90%; relapse and clonal evolution occur at rates that are comparable to what is observed in patients of all ages [47,50,51].

Both refractory and relapsed patients are frequently treated with further courses of ATG. In these settings, rabbit ATG (r-ATG) + CsA has been frequently used. R-ATG is similar to h-ATG except that gamma immune globulin is obtained by immunization of rabbits with human thymocytes. Clinically, r-ATG appears to be more immunosuppressive as a more prolonged lymphopenia is observed with this agent compared to h-ATG [52]. This enhanced lymphocytotoxicity of r-ATG may be explained by higher affinity IgG subtype to human lymphocytes, less batch-to-batch variability, longer half-life, and more efficient lymphocyte depletion [53]. In addition, r-ATG may promote immune regulation as suggested by an in vitro assay where CD4+CD25⁻ were converted to CD4+CD25⁺ regulatory T cells in the presence of r-ATG but not h-ATG [54]. For the 1/3 of patients who are refractory to h-ATG/CsA, repeated courses of immunosuppression have yielded response rates varying from 30 – 70% [55–57]. For relapsed patients, re-introduction of CsA commonly result in improvement in blood counts, however, CsA-dependence is frequent and the dose of CsA usually is tapered slowly with hematologic monitoring [45]. Re-treatment with ATG/CsA in relapsed AA has resulted in response rates of 50–60% [56–58]. In our experience, relapse

does not correlate to a poor prognosis, as patients often respond to re-introduction of CsA and/or re-treatment with ATG.

In contrast, patients refractory to initial h-ATG historically have had a dire outcome, with long term survival rates in the 1990s of 20–30% [45]. However, we have noted a striking improvement in survival among non-responders to initial h-ATG treated at our institution in recent years [59]. Retrospective analysis has shown that the decrease in deaths in refractory patients is most likely due to more successful salvage therapies (repeat IST and HSCT) as well as better supportive care, mainly the introduction of better antifungal drugs.

Despite better understanding of the pathogenesis of SAA, methods to predict response to IST are lacking. Proposed criteria to date have been complex or relied on non-standardized tests. A recently completed retrospective analysis of over 300 patients treated with h-ATG/CsA at our institution showed that baseline absolute reticulocyte count (ARC) and absolute lymphocyte count (ALC) combined served as a good predictor of response to IST [60]: patients with an ARC $\geq 25,000$ and ALC $\geq 1,000$ / μ L at baseline had an 80% response rate compared to 40% with those with an ARC $< 25,000$ and ALC $< 1,000$ / μ L. Age less than 18 years also correlated to improved response (about 75%). Since the addition of CsA to h-ATG, efforts to improve beyond h-ATG/CsA have been frustrating. The addition of a third immunosuppressive drug to the h-ATG + CsA standard regimen (mycophenolate mofetil, sirolimus, androgens, corticosteroids) have not resulted in better response rates or decreased relapse rates and clonal evolution [47,61–63]; and more potent lymphocytotoxic agents have been associated with unacceptable toxicities [64]. The role of G-CSF in adjunction to ATG + CsA remains controversial. A recent reported Japanese randomized study suggested that the addition of G-CSF might reduce the incidence of relapse [65], but this results was not observed in other studies of similar design [66]. In addition, a higher incidence of evolution has been reported in AA patients who receive G-CSF [37]. A large multicenter randomized European trial is under way which compares ATG and CsA with or without G-CSF which will more definitively address the benefits and possible pitfalls of cytokine addition in SAA.

In moderate AA, the clinical course is variable: some patients progress to severe disease, others remain stable and may not require intervention; regular transfusions may not be required [67]. Very few clinical trials have specifically addressed moderate disease. Immunosuppression can reverse moderate pancytopenia and alleviate transfusion requirements; ATG and cyclosporine are more effective in combination [68]. Daclizumab, a humanized monoclonal antibody to the interleukin-2 receptor, improved blood counts and relieved transfusion requirements in 6 of 16 evaluable patients; the outpatient regimen had little toxicity [69].

Allogeneic Hematopoietic Stem Cell Transplantation

HLA-matched sibling donor transplant

Allogeneic bone marrow transplantation from a histocompatible matched sibling is curative therapy in the majority of SAA patients who undergo this procedure. The most recent cohort reported to the IBMTR showed 77% 5-year survival [70], and in children and patients who were minimally transfused, survival of 80–90% may be routinely achieved [71]. Acute grade II–IV graft-versus-host-disease (GVHD) occurs in about 20–30% of patients and chronic GVHD in 30–40% [71,72]. Chronic GVHD has been a major cause of morbidity and mortality in patients who survived more than 2 years post graft, with the necessity of long term immunosuppressive therapy common [72]. Graft rejection, a historic problem in the application of transplant to SAA is now infrequent, likely a benefit of less immunogenic blood products (leukocyte-depleted erythrocytes, for example) from fewer donors (platelets collected by cytoapheresis) [73,74]. Also, better radiation-free conditioning regimens have

improved the tolerability of HSCT and allowed for engraftment in heavily transfused (and in some cases alloimmunized) patients who were refractory to IST [75,76]. The frequently employed conditioning regimen of cyclophosphamide + ATG was compared to cyclophosphamide in a prospective randomized study [77]: no differences in engraftment, GVHD, and survival rates were observed between the two groups, suggesting that the better outcome with HSCT over time relates to advances in supportive care. The main source of stem cells up to 2000 has been bone marrow cells; in recent years, G-CSF peripheral blood (PB) mobilized CD34+ have become more widely used. In a retrospective analysis, the use of PB progenitor cell graft has been correlated to a worse outcome and more chronic GVHD in younger patients (less than 20) compared to those who receive a bone marrow graft in HLA-matched sibling donor transplants [78]. This difference was not observed in older patients.

Matched unrelated donor transplant

A matched sibling donor is available in only 20–30% of cases. As the outcome in aplastic patients who have failed a single round of ATG has been poor, alternative sources of hematopoietic stem cells have been sought, usually from now very large donor registries. Data from large retrospective studies suggest that the outcome for an unrelated donor HSCT remains less favorable compared to a matched-related transplant, due to more GVHD, a mortality rate that is about twice that observed in matched sibling transplants, and long term survival of about 50% [70,79–81]. Older patients with poor performance status have the worst outcome and better results are obtained in children than for adults. Progress in donor selection through high-resolution HLA typing technology has likely contributed to decreased graft rejection and better survival [82] and recently reported outcomes for MUD rival those for an HLA-matched sibling transplant in children and young adults [83,84].

The optimal conditioning regimen for a MUD HSCT remains uncertain; in contrast to allogeneic sibling transplants, **transplants from unrelated donors still require low dose irradiation (200 cGy) to ensure engraftment, with attendant long-term complications** [85,86]. In general, prospective trials have enrolled fewer patients but have better results, albeit with short periods of post-transplant observation [71]. A multicenter prospective study compared the outcomes of a second course of IST to a MUD HSCT in children who failed initial course of IST: among the 60 initial ATG failures, 21 underwent a MUD HSCT and 31 received a second course of IST. Those who underwent a MUD HSCT had a higher failure free survival (defined as survival with response) compared to those who underwent a second course of IST, although no difference in overall survival was observed between the 2 groups [87].

Conclusion

In recent years, further evidence has accumulated to strengthen the hypothesis that bone marrow failure in AA results from immunologic destruction of hematopoietic stem and progenitor cells. In a minority of patients with shortened telomeres, a qualitative disorder may accompany the numerical diminution of CD34+ cells in the bone marrow. Current research is aimed at investigating the mechanisms that lead to T cell activation and, whether it is antigen driven or a result of immunological disarray. The clinical implication of telomere shortening in AA is being investigated for prognostic significance. Most patients with AA are now expected to survive regardless of the treatment modality employed. Advances in supportive care and better salvage therapies have produced significantly increased survival among initial non-responders to ATG+ CsA; the decrease in allo-sensitization (through routine leucodepletion of blood products) and better donor selection (through high molecular HLA-typing) have improved the outcomes of both related and unrelated HSCT; long term survival in pediatric patients who respond to IST is excellent and

ATG based regimen should be offered as initial therapy to those who lack a matched sibling donor. Our practice has been to consider an alternative donor HSCT in suitable pediatric patients who have failed an initial course of ATG + CSA or in adults who have failed two courses of IST. Refractory patients who are not suitable candidates for alternative donor HSCT can be supported through a combination of transfusions, androgens and/or administration of hematopoietic growth factors. In chronically transfused patients, iron overload can now be managed with more convenient oral agents (deferasirox, Exjade®) and, the prospect of approval of newer oral thrombopoietic drugs is likely to ease platelet transfusion requirements especially in AA patients who are refractory to IST.

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