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The Immune-mediated Pathogenesis and Treatment of Aplastic Anemia

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Abstract Aplastic anemia is an immune-mediated disorder in most cases. The suppression of hematopoiesis by CD8+ T cells and also the overproduction of interferon(IFN)-γ and tumor necrosis factor (TNF)-α due to the Th1 response were demonstrated. Aplastic anemia patients with HLA-DRB1*1501 and oligoclonality of the T-cell repertoire in the peripheral blood showed immunosuppressive therapy (IST), cyclosporine A (CyA) dependence. A minor population of paroxysmal nocturnal hemoglobinuria (PNH)-type blood cells, CD55-CD59-, predicts the response to IST. In patients with aplastic anemia, CD3+,CD4+ IL-17-producing T (Th17) cells were increased in the peripheral blood, whereas CD4+,CD25+,Foxp3+ regulatory T (T_{reg}) cells, were decreased, suggesting the regulation of hematopoiesis by these cells in aplastic anemia.

The standard immunosuppressive regimen for aplastic anemia has been horse antithymocyte globulin (hATG) and CyA, generating a hematologic response in 60-70% of patients receiving it as the first-line therapy. However, hATG was withdrawn from the market, and rabbit ATG (rATG) is the only formulation currently available in Japan. There are only limited data on rATG as the first-line therapy, and the clinical efficiency of rATG has not been established. Relapse after the initial response to IST is frequent, 30-50%. A very slow tapering of CyA is recommended. Hematopoietic stem cell transplantation (HSCT) is the treatment with the highest probability of cure in patients under 40 years old.

Key words: aplastic anemia, immune-mediated pathogenesis, treatment

Introduction

Primary bone marrow failure syndromes (BMFS) are a heterogeneous group of disorders characterized by the impaired production of one or more of the blood cell lineages, including aplastic anemia, myelodysplastic syndrome (MDS), and paroxysmal nocturnal hemoglobinuria (PNH). A typical example of BMFS is aplastic anemia. Recent advances in studies on the pathogenesis of aplastic anemia and treatment were reviewed.

Immune-mediated pathogenesis of aplastic anemia

Aplastic anemia is an immune-mediated disorder caused in most cases by the following diverse phenomena. The pathophysiology and treatment of aplastic anemia were extensively studied by Young and colleagues and reviewed.[1]

Zoumbos et al. demonstrated the suppression of hematopoiesis by suppressor CD8+ T cells and also the overproduction of interferon-γ (IFN-γ) and tumor necrosis factor (TNF)-α by a patient's T cells.[2,3] We also demonstrated the increased production of TNF-α by mononuclear cells,[4] and increased

soluble TNF-receptor in the plasma of aplastic anemia patients.[5] These results demonstrated that hematopoietic cells are destroyed through Th1 cells. Recently, Kordasti et al. demonstrated that not only Th1 cells but also IL-4- producing CD4+ T cells (Th2 cells) are expanded in aplastic anemia patients.[6]

Nakao et al. demonstrated that aplastic anemia patients possessing HLA-DRB1*1501 form a distinct subset of immune-mediated aplastic anemia.[7] The lymphocyte fraction expressing HLA-DRB1*1501 diminishes according to the improvement of aplastic anemia with cyclosporine A (CyA), but also increases with the relapse of aplastic anemia associated with the dose reduction of CyA, and, therefore, immune mechanisms through T cells are most likely to operate in these patients. It is hypothesized that some antigens likely to be presented to HLA-DR15-sensitized T cells lead to the attack of hematopoietic cells in these patients.[7] The frequency of aplastic anemia patients requiring continuous CyA therapy among aplastic anemia patients is estimated to be approximately 15%.[7] Zeng et al. demonstrated the oligoclonality of the T-cell repertoire indicating the involvement of the antigen-driven T-cell response in aplastic anemia patients who needed the continuous administration of CyA to maintain the remission of aplastic anemia.[8]

Sugimori et al. demonstrated that a minor population of PNH-type blood cells, CD55-CD59-, predicts the response to IST and a favorable prognosis in patients with aplastic anemia.[9] The results indicate the escape of PNH-type hematopoietic stem cells from immune attack by cytotoxic T cells, and the clonal expansion of these cells at the onset of aplastic anemia.

Katagiri et al. demonstrated that the frequent loss of HLA alleles, HLA-A*02:01, A*02:06, A*31:01, and B*40:02, associated with 6p loss of heterozygosity (LOH)was observed in acquired aplastic anemia patients.[10] This finding also indicates that 6p LOH (+) hematopoiesis found in aplastic anemia patients represents the escape of suppression of hematopoiesis from autoimmunity, which is mediated by cytotoxic T cells that target the relevant auto-antigens presented on hematopoietic progenitors though these class I HLAs.

Maciejewski et al. demonstrated the increased expression of Fas antigen on bone marrow CD34+ cells in aplastic anemia, indicating the mechanism of damage through the Fas/Fas-Receptor apoptotic pathway.[11] However, we observed the decreased expression of the Fas ligand on peripheral blood mononuclear cells, and undetectable levels of the soluble Fas ligand in the serum of patients with aplastic anemia and MDS.[12]

Th17-mediated immune response and regulatory T cells in aplastic anemia

CD3+,CD4+ IL-17-producing T (Th17) cells, which are distinct from Th1 or Th2 cells, have been shown to play a crucial role in autoimmune tissue injury.[13] Whereas, CD4+CD25+Foxp3+ regulatory T (T_{reg}) cells inhibit autoimmunity and protect against tissue injury.[13]

Transforming growth factor (TGF)-β is a critical differentiation factor for the generation of T_{reg} cells. Instead, IL-6 completely inhibits the generation of T_{reg} cells induced by TGF-β. Furthermore, IL-6 and TGF-β together induce the differentiation of Th17 cells from naïve T cells.[13]

de Lantour et al. demonstrated that the frequency and total number of Th17 cells was increased in patients with aplastic anemia,[14] being correlated with disease activity. Kordasti et al. also observed more markedly increased Th17 cells in severe than in non-severe aplastic anemia.[6] In contrast, Solomou et al. observed that T_{reg} cells were decreased at disease presentation in patients with aplastic anemia.[15] Thus, there was an inverse relationship between the number of Th17 cells and T_{reg} cells. Kordasti et al. also observed decreased activated and resting T_{reg} cells, and T_{reg} cells from aplastic anemia patients were unable to suppress normal effector T cells, while effector T cells of aplastic anemia patients were suppressed by normal T_{reg} cells; thus, T_{reg} cells of aplastic anemia patients were functionally abnormal.[6] Gu et al. also observed that IL-17 prompted macrophages to produce cytokines of IL-8, IL-6, and TNF-α in aplastic anemia patients.[16] Thus, the Th17 immune response contributes to the pathophysiology of aplastic anemia,

especially at the beginning of the disease.

Interleukin-27 in aplastic anemia

Li et al. reported that the mRNA expression of interleukin-27 (IL-27)/IL-27R subunits of the IL-12 family in the bone marrow nuclear cells and level of IL-27 in the bone marrow plasma were increased.[17] IL-27 enhanced the production of TNF-α and IFN-γ by the bone marrow mononuclear cells in aplastic anemia.[17] These data suggest that IL-27 and IL-27-induced TNF-α and IFN-γ overproduction might be involved in the pathogenesis of aplastic anemia.

Immunosuppressive therapies (IST) in the management of immune-mediated aplastic anemia

The treatment strategy for severe aplastic anemia is shown in Fig. 1. Over the last 2 decades, the standard immunosuppressive regimen for aplastic anemia patients has been anti-thymocyte globulin (ATG) and CyA.

Anti-thymocyte globulin (ATG)

ATG is a heterogeneous anti-serum obtained by injecting human lymphocytes into animals. Various ATG preparations exist, which differ in stimulating antigens (peripheral lymphocytes, thymocytes, or T-cell lines), and in the host animal (horse or rabbit). Horse anti-thymocyte globulin (hATG, Atgam, Pfizer), which is used almost exclusively in the USA, is different from the hATG preparation used in Europe and Japan (Lymphoglobulin, Genzyme). Horse ATG (hATG) plus CyA, the most well-studied regimen, generated a hematologic response in 60-70% of patients when used as the first-line therapy.[18-20] Rabbit antithmocyte globulin (rATG, Thymoglobulin, formerly Gen-

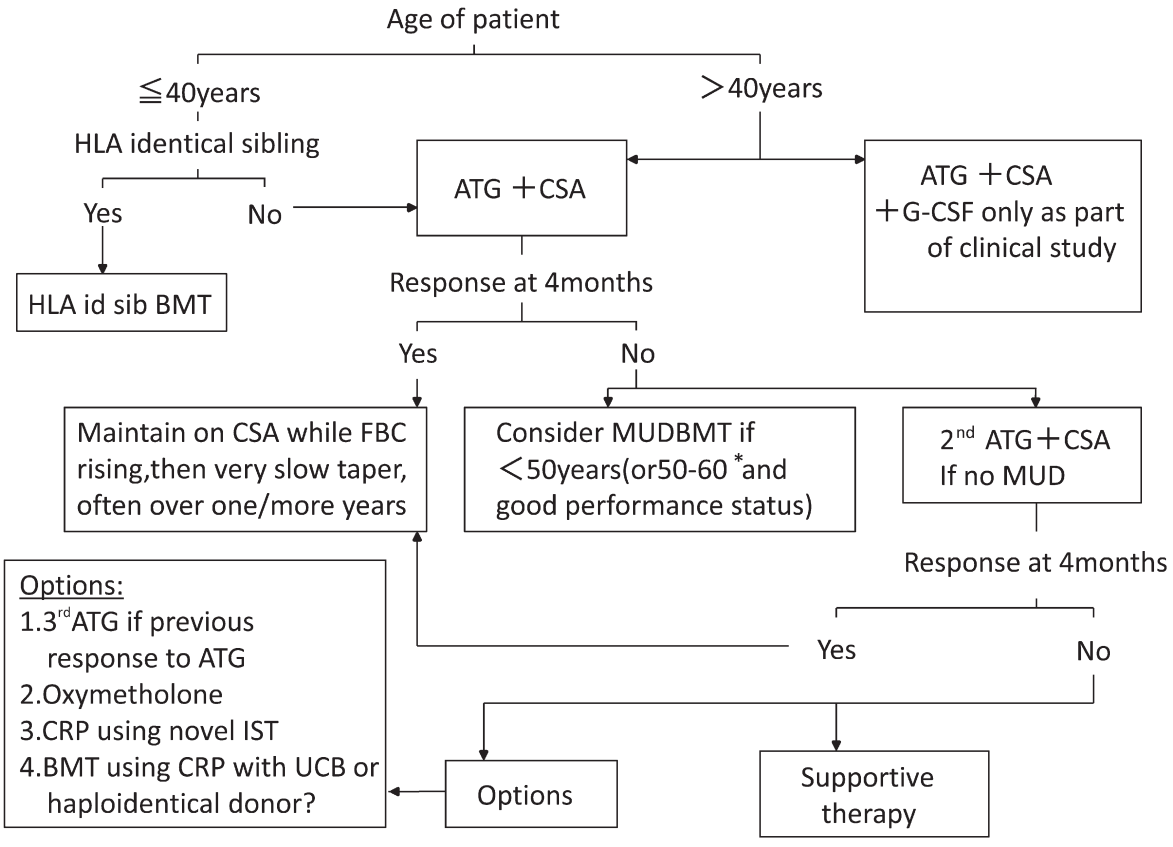

Fig. 1 Treatment strategy for severe aplastic anemia. Referred from reference 22. IST, immunosuppressive therapy; ATG, antithymocyte globulin; CSA, cyclosporine A; G-CSF, granulocyte-colony-stumulating factor; BMT, bone marrow transplantation; MUDBT, matched unrelated donor; UCB, umbilical cord blood; FBC, full blood count; CRP, clinical research protocol.

zyme, and presently Sanofi Aventis) was reserved for second-line therapy after failure of response or relapse after treatment with hATG. However, hATG (Lymphoglobulin, Genzyme) was withdrawn from the market in 2007, and rATG is the only formulation currently available in Europe and Japan. Alternative rATG forms can be obtained: two rATG are currently available (Thymoglobulin and ATG-Fresenius). A recent study demonstrated that rATG induced the expansion of functional T regs, but hATG did not.[21] These functional differences between rATG and hATG may have a clinical impact.

Clinical results of IST

A hematological response is usually seen within 3 or possibly 6 months from IST. Aplastic anemia patients with an HLA-DR 15 and PNH granulocyte population are associated with a higher response probability.[7,9] The expected probability of a response was 60-80% for the 5-year survival, with an overall survival of 75-85%.[22] Recently, Scheinberg et al. reported an unexpected difference in the rate of a hematological response at 6 months in favor of hATG plus CyA (68%) as compared with rATG plus CyA (37%).[19] The overall survival at 3 years also differed, with a survival rate of 96% using hATG as compared with 76% using rATG.[20] There are only limited published data on rATG (Thymoglobulin) as first-line immunosuppressive therapy in aplastic anemia. The clinical results with these rATG have not been established due to the lack of large randomized trials. Rabbit ATG was previously used in patients not responding to a first course of IST, or in patients relapsing after hATG, with response rates of 30-77%. The efficacy of rATG plus CyA as a first line was disappointing, with a lower hematologic response rate compared to that using hATG plus CyA.[23] The Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT SAAWP) reported similar results using rATG plus CyA in patients with severe or transfusion-dependent non-severe aplastic anemia.[18] In conclusion, most studies have shown significantly superior response, survival, and death rates with hATG compared with rATG.

In Japan, ATG plus CyA yielded a hematological response of 77 vs. 55% in patients with or without granulocyte colony-stimulating factor (G-CSF), respectively.[24] The relapse rate was lower in the group with than in that without G-CSF (42 vs. 15% at 4 years, respectively).

Many patients require long-term maintenance of IST by CyA to sustain their response, CyA-dependence, ranging between 25 and 50% of patients.[18]

Relapse of aplastic anemia after response to IST

Relapse after the initial response to IST is frequent, and about 30-50% of cases relapse within months or years after IST discontinuation.[18] Therefore, a very slow tapering of CyA is recommended, for example, by 25 mg every 3 months, or by 10% every month, and CyA should be continued for at least 12 months after a maximum response is obtained.

A second course of IST after relapse

A second course of IST is recommended if there is no response and is administered after 3 months, or there is relapse after the first course.[22] The treatment consists of either two courses of hATG plus CyA or subsequent rATG plus CyA. However, a recent study from Brazil indicated that rATG might not be as effective as hATG.[21] When rATG was given for the second course following an initial course of hATG, the response rate was 30% for non-responders and 65% for relapsed patients. A comparative study of the efficacy of hATG versus rATG has not yet been conducted in Japan. Alternatively, a second IST could be attempted before proceeding to HSCT. Patients relapsing after an initial response to IST are likely to respond to a further IST course (but are likely to never be cured); thus, the choice between additional IST and the HSCT procedure may be difficult. The quality and duration of the first response to IST may be informative in predicting the hematological response to further IST.

Failure of IST

Failure of IST is observed in 20-30% of pa-

tients.[16] There are several possibilities for the failure of IST. The first is that drugs are unable to eradicate the immune attack. The second is that, although IST is effective for controlling the immune attack, the hematopoietic stem cells (HSC) have already been exhausted. The third is that the mechanism of aplastic anemia is not immune-mediated. Furthermore, clonal evolution, including progression to MDS and acute myelogenous leukemia (AML), or the emergence of a PNH clone, accounts for about 10-15% of treatment failure.

Novel candidates for immunosuppressive regimens

There are several novel candidates for immunosuppressive regimens.[18] Humanized anti-CD52 antibody, alemtuzumab (CampathIH), was effective in relapsed and refractory severe aplastic anemia, but not in treatment-naïve patients.[25]

To improve the suppression of hematopoiesis, several kinds of cytokines have been evaluated: etanercept (a TNF-receptor Ig fusion protein, Enbrel), infliximab (a chimeric anti-TNFα mAb, Remicade), adalimumab (a fully humanized anti-TNF-α mAb), and fontolizumab (an anti-IFN-γ mAb).[18,26]

Recently, a long term-follow-up study of a human IgG1 monoclonal antibody specific to the α subunit of IL-2 receptor, daclizumab, reportedly led to a hematological response in patients with aplastic anemia and also improved cytopenia in MDS.[27]

These studies were performed in small trials, and their clinical efficacies were not fully evaluated.

The apoptosis of progenitor cells

In patients with lower-risk MDS (International Prognostic Scoring System, IPSS, low and intermediate-1), the same pathophysiology as in aplastic anemia was suggested; therefore, IST may also be effective for the hematological improvement of lower- risk MDS.[27] The apoptosis of progenitor cells in low-risk disease is mediated through other (pro)-inflammatory cytokines, such as TNF-α, IL-1, IL-2, IL-6, IL-12, and IFN-γ. TNF-α is a pro-inflammatory cytokine that plays a crucial role in the activation of cellular immunity. In the absence of TNF-α, naïve CD4+T cells may differentiate, under the influence of TGF-β, into T_{reg} rather than Th17 cells. Enhanced T_{reg} differentiation may, therefore ,be one of the mechanisms by which TNF-α therapy suppresses the cellular immune response. Anti-TNF-α, TNF-related apoptosis inducing ligand (TRAIL) inhibitors and anti-IL-2 cytokine therapies provide other immunotherapeutic approaches to deal with apoptosis in low-risk MDS.[27]

Hematopoietic stem cell transplantation (HSCT)

HSCT is the treatment strategy with the highest probability of curing aplastic anemia.[18,22] For patients with HLA-matched sibling donors, HSCT is the best initial treatment in young adults (<40 years old). All other patients (no sibling donor or >40 years old) should initially receive an IST course, and HSCT should be considered in the case of IST failure. For patients lacking a suitable donor, a second IST is the next choice after initial IST failure. Patients below 60 years who lack a response to a second IST should be considered for HSCT from the best-matched donor available. In aplastic anemia, bone marrow stem cells are recommended as the source of hematopoietic stem cells for transplantation. An experimental HSCT setting includes any of the following: >60 years, haploidentical donor, and cord blood stem cells; and these should be performed in the setting of prospective clinical trials.

Conflict of Interest

The author states no conflict of interest.

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