Acquired Aplastic Anemia

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Acquired aplastic anemia (AA) is a disorder characterized by a profound deficit of hematopoietic stem and progenitor cells, bone marrow hypocellularity, and peripheral blood pancytopenia. It primarily affects children, young adults, and those over 60 years of age. The majority of cases are idiopathic; however, idiosyncratic reactions to some drugs, chemicals, and viruses have been implicated in its etiology. An autoimmune T-cell reaction likely causes the stem cell depletion, but the precise mechanism, as well as the eliciting and target antigens, is unknown. Symptoms vary from severe life-threatening cytopenias to moderate or non-severe disease that does not require transfusion support. The peripheral blood typically exhibits pancytopenia, reticulocytopenia, and normocytic or macrocytic erythrocytes. The bone marrow is hypocellular and may exhibit dysplasia of the erythrocyte precursors. First line treatment for severe AA consists of hematopoietic stem cell transplantation in young patients with HLA identical siblings, while immunosuppression therapy is used for older patients and for those of any age who lack a HLA matched donor. Patients with AA have an increased risk of developing paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS), or acute leukemia. Further elucidation of the pathophysiology of this disease will result in a better understanding of the interrelationship among AA, PNH, and MDS, and may lead to novel targeted therapies.

ABBREVIATIONS: AA = acquired aplastic anemia; IST = immunosuppression therapy; PNH = paroxysmal nocturnal hemoglobinuria; MDS = myelodysplastic syndrome.

INDEX TERMS: acquired aplastic anemia; myelodysplastic syndrome; paroxysmal nocturnal hemoglobinuria.

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Acquired aplastic anemia (AA) is a bone marrow failure disorder characterized by a marked reduction in the number of hematopoietic stem cells, hypocellular bone marrow, and peripheral blood pancytopenia. The resulting decreased levels of circulating platelets, erythrocytes, and granulocytes can cause life-threatening symptoms of bleeding, anemia, and later in the course of disease, infection. Acquired AA is rare, with an incidence of two per million in Europe and North America, but a two to three-fold greater incidence in East Asia.[1,2] There are no significant gender differences, and the peak age distribution is bimodal at 10 to 25 years and over 60 years of age.[2]

ETIOLOGY

Cytotoxic drugs and radiation therapy cause bone marrow hypoplasia and cytopenia that is anticipated, usually predictable, and dose dependent.[1] The bone marrow and blood cell counts recover upon discontinuation. In contrast, acquired AA is bone marrow failure in which the cause is largely idiopathic or unknown. Approximately 15% to 25% of acquired AA may occur as idiosyncratic reactions to various drugs, or after exposure to certain chemicals or viruses.[1,2] Idiosyncratic reactions are rare, unexpected, and unpredictable, and the bone marrow does not usually recover after the agent is withdrawn. Table 1 lists some agents associated with

acquired AA.[1,2,3,4] The incidence of AA as a complication of drug therapy is rare. A genetic predisposition to idiosyncratic reactions may exist due to individual differences in metabolic or immune response pathways. Sutton and others found a higher than expected frequency of glutathione S-transferase (GST) gene deletions in AA. GSTT1 null and GSTM1/GSTT1 double null genotypes were found in 30% and 22% of Caucasians with AA, respectively.[5] GST deficiency may hinder the biometabolism of some chemical toxins and increase the risk of development of AA.[5]

AA may occur as a rare complication in pregnancy, autoimmune disease, and in some viral infections, such as Epstein-Barr virus and human immunodeficiency virus.[1,2] As many as 2% to 10% of patients have a history of acute hepatitis one to three months prior to presentation with severe AA; however, tests for hepatitis A, B, and C are negative.[6] It is important to recognize hepatitis-associated AA syndrome for its poor prognosis and high mortality rate.

Quantitative and qualitative deficiency of hematopoietic stem and progenitor cells cause the peripheral blood pancytopenia and hypocellular bone marrow in acquired AA.[7,8] In aplasia following cytotoxic chemotherapy and radiation, as well as in benzene toxicity, there is direct damage to the DNA or proteins of hematopoietic stem cells and progenitor cells, resulting in cell death.[1] However, in idiopathic AA, viral associations, and idiosyncratic reactions to drugs or chemicals, bone marrow failure is thought to be the result of a T-cell-mediated autoimmune attack against hematopoietic stem and progenitor cells.[9] It is likely that the offending antigen in this autoimmune reaction is expressed on stem and early progenitor cells, but the precise mechanism for this reaction, as well as the identity of the inciting and target antigens, remains unknown.

Table 1. Agents infrequently associated with acquired aplastic anemia

Drugs
Nonsteroidal anti-inflammatory agents (butazones, indomethacin, diclofinac, naproxen, piroxicam)
Antiarthritics (gold salts, D-penicillamine)
Antithyroids (carbimazole, thiouracil)
Antibiotics (chloramphenicol, sulphonamides)
Anticonvulsants (carbamazepine, phenytoin)
Antidepressants (phenothiazine, dothiepin)
Antiprotozoals (chloroquine, quinacrine)
Antidiabetes drugs (chlorpropamide, tolbutamide)
Occupational and environmental exposures
Benzene*
Insecticides
Petrochemicals
Lubricating agents

* Benzene is more commonly associated with acquired AA than the other agents listed, but the incidence varies by the amount and duration of exposure. The incidence of acquired AA is estimated at 1 in 10,000 individuals exposed at air concentrations of 10 to 20 ppm, and increases to 1 in 100 at concentrations >100 ppm.[4]

PATHOPHYSIOLOGY

Hematopoietic stem cells are severely decreased in the bone marrow of AA patients as evidenced by a greater than ten-fold reduction in CD34+ cells detected by flow cytometry (mean of 557/mL in AA compared to 5,867/mL in normal controls), and diminished colony formation and growth of long-term culture initiating cells in vitro.[7,8] Stem cell numbers remain low despite elevated serum growth factors.[10] Stromal cells in AA produce normal or increased growth factors and support the growth of normal CD34+ cells in culture.[10,11,12] Further evidence of a functional stroma is demonstrated clinically by the successful engraftment of bone marrow transplants in AA patients.

An autoimmune pathophysiology of AA was first proposed in the late 1960s when patients had an unexpected improvement in cell counts after unsuccessful marrow transplantation.[13] Mathe and others suggested that the improvement might be due to the antilymphocyte globulin used in the immunosuppressive conditioning regimen required for transplant. Since then, there has been increasing laboratory and clinical evidence in support of an autoimmune pathophysiology of the bone marrow failure. This is indirectly inferred from the fact that the majority of patients with AA respond to immunosuppressive therapy with improvement of cell counts.[2] In laboratory experiments, bone marrow cells from AA patients inhibit hematopoietic colony formation.[14] Furthermore, T-cells in acquired AA produce increased amounts

of interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α), known inhibitors of hematopoiesis.[15,16] In AA patients, there is an increase in the number of cytotoxic CD8+ cells in the blood and bone marrow, as well as an increase in their expression of the HLA-DR activation marker and cytoplasmic INF-γ measured by flow cytometry.[17] These findings were shown to reverse after immunosuppressive therapy.[17] Nimer and others found that HLA-DR2 has a 1.9-fold higher incidence in AA than in the general population.[18] However, the significance of that finding in terms of pathophysiology, treatment, or prognosis is unclear.

Patients with AA have a greater proportion of apoptotic CD34+ cells compared to normal controls when stained with fluorescent 7-amino actinomycin D and measured by flow cytometry, and these cells have a higher expression of Fas receptors.[19,20] Since INF-γ and TNF-α induce apoptosis through Fas receptor signaling, this pathway may be important in the destruction of stem cells in AA.[20] Further, gene expression profiling of CD34+ cells using GeneChip analysis demonstrates a marked increase in expression of apoptotic genes in AA compared to normal individuals.[21]

Various mechanisms may initiate the autoimmune response in AA such as:[9,17]

- antigen modification by drugs, chemicals or their metabolites,
- alteration of self-proteins,
- viral-induced aberrant protein expression,
- production of novel fusion proteins due to undetected chromosome alterations,
- cross-reactivity of drugs, chemicals, or viruses with self antigens, or
- exposure of cryptic antigens by tissue damage.

The offending antigen in acquired AA may also be a membrane glycosylphosphatidylinositol (GPI)-anchored protein or the GPI anchor itself.[22,23] Approximately one-third of AA patients experience an expansion of clones that lack the GPI anchor and its associated proteins, the defect in paroxysmal nocturnal hemoglobinuria (PNH).[22] GPI-negative PNH clones are able to proliferate in the marrow of acquired AA patients and apparently are not affected by the immune destruction and apoptosis experienced by the other progenitor cells in the marrow.[22]

Table 2. Peripheral blood findings in acquired aplastic anemia

Decreased
Absolute neutrophil count
Hemoglobin
Platelet count
Reticulocyte count
Reticulocyte production index
White blood cell count
Increased or normal
Mean cell volume
Blood smear
Decreased neutrophils, monocytes, and platelets
Normocytic or macrocytic red blood cells
Toxic granulation of neutrophils may be present

CLINICAL FINDINGS

Severe AA can be rapidly fatal, while the clinical course of non-severe aplastic anemia may be asymptomatic and transfusion-independent.[2] Patients with AA do not exhibit splenomegaly and hepatomegaly. The most common symptoms at presentation are bleeding due to thrombocytopenia and fatigue, dyspnea, and pallor due to anemia. Bruising, petechiae, epistaxis, bleeding gums, excessive menses, retinal hemorrhages, intestinal bleeding, and rarely cerebral hemorrhage may occur as manifestations of the thrombocytopenia. Fever and infection, due to neutropenia, are infrequent at presentation, but may occur later in the disease. Prolonged neutropenia can result in fatal bacterial sepsis and systemic fungal infections.[2,24]

LABORATORY FINDINGS

Peripheral blood

Table 2 summarizes the peripheral blood findings in acquired AA. Platelet, white blood cell, and red blood cell counts are decreased, but initially only one or two of the cell lines may be affected.[1] The hemoglobin is below 10 gm/dL, and the reticulocyte count and reticulocyte production index are decreased reflecting the inability of the bone marrow to adequately respond to the anemia. The absolute neutrophil count is decreased, but the absolute lymphocyte count is usually normal. The mean cell volume is normal or increased.

The peripheral blood film has decreased numbers of platelets, neutrophils, and monocytes. Blasts and bands are characteristically absent, and neutrophils may have toxic granulation. The red blood cells can be normocytic or macrocytic without other morphologic abnormalities. The platelets are normal in appearance.

Bone marrow

Bone marrow aspirates and biopsies are hypocellular with prominent fat cells, and patchy cellularity (Figure 1). A bone marrow biopsy is required for accurate assessment of cellularity.[2] Blast, granulocytic, and megakaryocytic cells are decreased or absent, and reticulin staining is normal. Lymphocytes, plasma cells, and macrophages may be present. Although dyserythropoiesis may be found, there is no dysplasia of the granulocytes or megakaryocytes.

Figure 1. Hypocellular bone marrow aspirate in aplastic anemia, 100X magnification, Tetrachrome-Giemsa stain. Note prominent fat cells.

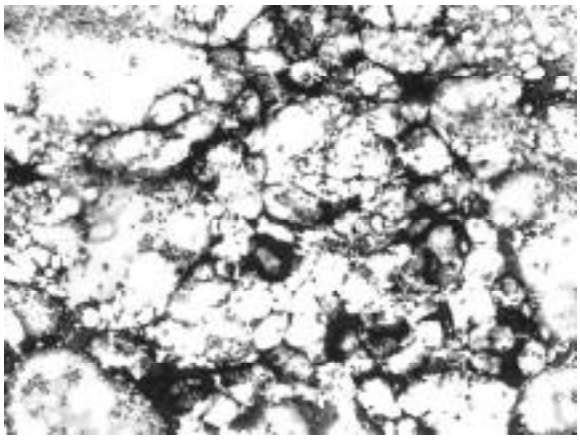

Photo courtesy of Dr Peter Maslak, Memorial Sloan Kettering Cancer Center, New York.

Classification

Acquired AA is classified as non-severe aplastic anemia (NSAA), also called moderate aplastic anemia (MAA), severe aplastic anemia (SAA), and very severe aplastic anemia (VSAA) based on cell counts and bone marrow cellularity. Table 3 depicts the criteria for each category.[25,26] Classification of AA patients by severity of disease is important to guide treatment decisions.

Other laboratory findings

There is a marked decrease in CD34+ cells measured by flow cytometry, and their quantity may remain low even after hematopoietic recovery.[7,8] Serum levels of erythropoietin and other growth factors are elevated.[10] Liver function tests may be abnormal if the AA was preceded by acute hepatitis.[2]

Chromosomal abnormalities are infrequent in AA at presentation, and their development is considered by some as a reason to exclude a diagnosis of AA.[2,27,28] As many as 26% of AA patients develop an abnormal karyotype over the course of their disease.[27,29] The most common abnormalities found include abnormalities of chromosome 7, trisomy 8, and abnormalities of chromosome 13.[2,27,28] Chromosome abnormalities in some AA patients may disappear and reappear during the course of the disease, the significance of which is uncertain. Evaluation of chromosomes is often difficult since hypocellular bone marrows with decreased proliferation potential may yield few metaphases for analysis. Karyotyping of peripheral blood lymphocytes may be more successful.[2] The incidence of chromosome abnormalities may be higher with the use of more sensitive techniques such as the interphase fluorescent in situ hybridization (FISH) using probes for specific chromosomes.[2,5,29]

Differential diagnosis

It is important to distinguish AA from similar conditions so that the appropriate treatment can be implemented. PNH and AA may have similar features of pancytopenia, macrocytosis, and bone marrow hypocellularity; however, patients who present with primary PNH have reticulocytosis and clinical and biochemical evidence of hemolysis.[2] Due to an acquired clonal mutation, PNH cells lack the GPI anchor on their surface, and are characteristically deficient in GPI-linked proteins such as

Table 3. Classification of acquired aplastic anemia

Non-severe aplastic anemia (NSAA) or moderate aplastic anemia (MAA)
Presence of at least two of the following:
- Hemoglobin < 10 g/dL
- Platelets: 20-50 x 10^9/L
- Neutrophils 0.5-1.5 x 10^9/L

Severe aplastic anemia (SAA)
Bone marrow cellularity <25%, or 25% to 50% with <30% residual hematopoietic cells, and presence of at least two of the following:
- Neutrophils <0.5 x 10^9/L
- Platelets <20 x 10^9/L
- Reticulocytes <20 x 10^9/L

Very severe aplastic anemia (VSAA)
Includes criteria of SAA plus:
- Neutrophils <0.2 x 10^9/L

CD55 and CD59.[2,22] This leads to increased susceptibility to complement-mediated hemolysis. Flow cytometry is the most sensitive method for detection of PNH cells, and the classical sucrose hemolysis test and Ham test for complement-mediated hemolysis may be positive if a sufficient number of PNH erythrocytes are present in the peripheral blood.[2, 24]

There is a strong association between AA and PNH. Bone marrow failure is a major clinical manifestation of PNH [24,29]. Conversely, expansion of GPI-negative PNH clones and development of hemolytic PNH occurs in approximately 10% to 25% of AA patients. [22,29] Wang and others detected PNH neutrophils in the peripheral blood of 88.6% of newly diagnosed AA patients using a sensitive flow cytometric technique that analyzed at least 10^5 cells in the granulocyte gate.[30] The appearance of PNH cells in acquired AA is sometimes transient, and the significance of this finding is uncertain. AA and PNH may be a single entity that can present either as primary AA or primary PNH.[29] Nevertheless, it is important to test for PNH cells in acquired AA due to the complications of hemolysis and thrombosis associated with the disorder.[2]

Acquired AA is also similar to myelodysplastic syndrome (MDS) in that both can have pancytopenia, macrocytosis, and dyserythropoiesis.[2] Although the bone marrow in MDS is usually hypercellular, 20% of MDS cases have a hypocellular bone marrow at presentation.[1,2] MDS usually has chromosomal abnormalities, however, they may not always be present.[24,27] MDS has additional features that are absent from acquired AA including dyspoiesis of the granulocytic and megakaryocytic cells, increased blasts, and increased reticulin in the bone marrow.[2] Approximately 20% of patients with acquired AA progress to MDS during the course of their disease.[29] Although this progression is often preceded by the appearance of chromosome abnormalities, the underlying mechanism for the clonal evolution to MDS is unknown.[27,29]

AA may also be similar to hypocellular acute leukemia, however, acute leukemia has increased blasts and reticulin in the bone marrow.[2] Hairy cell leukemia (HCL) also has pancytopenia, but the bone marrow in HCL is fibrotic, the circulating hairy cells co-express CD20, CD11c, CD25, and CD103, and most patients have splenomegaly.[2] Decreased levels of vitamin B_{12} and/or folate can identify the pancytopenia associated with megaloblastic anemia. Finally, acquired AA may be similar to Fanconi anemia, a rare congenital aplasia most often diagnosed in childhood, but occasionally presenting in adults.[2] A genetic test for mitomycin-C induced chromosome breakage is characteristic of Fanconi anemia.[1,24]

TREATMENT

The treatment rationale in acquired AA is twofold, either 1) replacement of the deficient or damaged stem cells in the bone marrow by transplantation, or 2) suppression of the autoimmune reaction against the stem cells. Hematopoietic stem cell transplantation (HSCT) is the preferred therapy for patients under 30 to 40 years of age that have a HLA identical sibling.[2,24] For approximately 70% of SAA patients, HSCT is not an option because of age or the lack of an appropriate donor. In these cases immunosuppression therapy (IST) is the standard initial treatment.[2,24] The purpose of IST is to prevent the T-cell attack on the hematopoietic cells by decreasing the number of activated T-cells and inhibiting their function.[31] Combined IST using antithymocyte globulin (ATG) and cyclosporine (CSA) is preferred since the combination has a greater response rate than either agent alone.[2,31] ATG results in the cytolysis and reduction of T-cells by recognition of their surface antigens, while CSA inhibits T cell activation and cytokine release.[31] IST may take months or years for improvement in cytopenias and independence from transfusions, and relapses are frequent. [31] Some patients may need to continue CSA therapy for a prolonged period, and even after a hematologic recovery, stem cell numbers remain low.[2,31,32] The reasons for the incomplete recovery with IST may be due to intrinsic defects in the stem cell, continued inhibition of the stem cells by lymphocytes, or irreversible stem cell loss.[32]

For those patients who are not initially responsive to IST, or those who relapse after treatment, a second or third IST course may be used.[2] If the patient is still not responsive, and the patient is young, HLA-matched unrelated bone marrow transplant may be an option; however, survival is not optimal with matched unrelated donors.[2]

Supportive care for thrombocytopenia and anemia is provided by platelet transfusions when the platelet count falls below 10 x 10^9/L (20 x 10^9/L in febrile patients) and red cell transfusions to alleviate anemia-related symptoms.[2] Antibiotics and antifungal drugs are used prophylactically in patients with prolonged neutropenia. The use of erythropoietin (rHuEPO) and other growth factors as primary treatments is not recommended due to lack of efficacy and serious side effects.[2,10,24] However, a short course of G-CSF may improve the neutrophil count in severely neutropenic patients with infections who are not responding to antibiotics.[2] Single agent corticosteroid therapy is not recommended because low doses are not effective and high doses result in excessive toxicity.[2,24]

Patients with NSAA who are transfusion-independent do not require treatment, but are periodically monitored for their blood cell counts and presence of abnormal cells.[2] IST is recommended once the patient becomes transfusion-dependent.

Other immunosuppressive agents under investigation for treatment of acquired AA include oxymetholone, ATG/CSA in combination with mycophenolate mofetil or G-CSF, rapamycin, and monoclonal antibodies to the IL-2 receptor. [2,24,33]

PROGNOSIS

Survival rate is poor when patients are treated only with transfusions and antibiotics.[24] Approximately 75% to 90% of young patients receiving HSCT from a HLA identical sibling have long-term survival.[24,29] Evolution of leukemia and myelodysplasia after HCST occurs infrequently, and is likely due to the conditioning regimen required for the transplant.[29]

Approximately 60% to 80% of patients respond to IST, and a functional cure is obtained in 50% of responding patients.[24,27,29,34] Approximately 10% to 25% of patients develop hemolytic PNH, and 10% to 20% progress to MDS or acute leukemia after IST.[22,29] One study reported a 42% combined risk of developing PNH or MDS.[35] AA patients who develop PNH have a better prognosis than those who develop MDS or leukemia. The development of MDS and acute leukemia is usually preceded by the development of chromosomal abnormalities. Patients who develop monosomy 7 have a higher likelihood of developing leukemia.[24,27] Patients with trisomy 8 have a better prognosis, but may require long term CSA to maintain normal counts.[24,27]

Peripheral blood granulocytes and monocytes of AA patients have a progressive shortening of their telomeres that may play a role in the evolution of MDS in these patients.[36]

CONCLUSION

Major progress has occurred in the elucidation of the pathophysiology of acquired AA as well as the protocols for its treatment. However, many questions remain about the triggering event for the autoimmune reaction, the inciting and target antigens, and the nature of the autoimmune response. Further research on the pathophysiology may lead to more specific therapeutic strategies and may further elucidate the interrelationship between acquired AA, PNH, and MDS.

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