Aplastic anaemia

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Lancet 2005; 365: 1647–56

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Aplastic anaemia is a rare haemopoietic stem-cell disorder that results in pancytopenia and hypocellular bone marrow. Although most cases are acquired, there are unusual inherited forms. The pathophysiology of acquired aplastic anaemia is immune mediated in most cases; autoreactive lymphocytes mediate the destruction of haemopoietic stem cells. Environmental exposures, such as to drugs, viruses, and toxins, are thought to trigger the aberrant immune response in some patients, but most cases are classified as idiopathic. Similarly to other autoimmune diseases, aplastic anaemia has a varied clinical course; some patients have mild symptoms that necessitate little or no therapy, whereas others present with life-threatening pancytopenia representing a medical emergency. Paroxysmal nocturnal haemoglobinuria and myelodysplastic syndrome commonly arise in patients with aplastic anaemia, showing a pathophysiological link between these disorders. Acquired aplastic anaemia can be effectively treated by allogeneic bone-marrow transplantation, immunosuppression (generally antithymocyte globulin and ciclosporin), and high-dose cyclophosphamide.

Aplastic anaemia is a rare, potentially life-threatening failure of haemopoiesis characterised by pancytopenia and bone-marrow aplasia. Ehrlich[1] described the first case in 1888, in a young woman who died of an abrupt illness with severe anaemia, haemorrhage, hyperpyrexia, and hypocellular bone marrow. In 1972, a patient with aplastic anaemia became the first recipient of successful allogeneic bone-marrow transplantation.[2] The development of bone-marrow transplantation and potent immunosuppressive therapy in the 1970s greatly improved the prognosis of an illness that had been fatal in most cases within a year of diagnosis. Although aplastic anaemia is still a potentially devastating illness, with prompt medical intervention most patients now survive it.

Inherited disorders of bone-marrow failure

This seminar focuses on the diagnosis, epidemiology, and treatment of acquired aplastic anaemia. This disorder can occur in any age-group, and in most cases results from an autoimmune attack against haemopoietic stem cells. Awareness of the rarer inherited forms of bone-marrow failure (table 1) is important. These disorders can masquerade as acquired aplastic anaemia but rarely respond to immunosuppressive therapies; management generally consists of supportive care or bone-marrow transplantation in severe cases.[3,4] Inherited bone-marrow failure generally presents in the first decade of life and is in many cases associated with physical anomalies (eg, short stature, arm anomalies, hypogonadism, and café-au-lait spots associated with Fanconi's anaemia). Some, but not all, of these patients have a positive family history of cytopenias; this feature highlights the importance of a careful family history in assessment of patients with aplastic anaemia.

Fanconi's anaemia, the most common form of inherited bone-marrow failure, is an autosomal recessive disorder that is characterised by defects in DNA repair and a predisposition to leukaemia and solid tumours.[5] An X-linked form of this disorder has lately been recognised.[6] Dyskeratosis congenita, an inherited bone-marrow-failure syndrome that classically presents with the triad of abnormal skin pigmentation, nail dystrophy, and mucosal leucoplakia, shows substantial clinical and genetic heterogeneity. X-linked recessive, autosomal dominant, and autosomal recessive forms are recognised.[7,8] The X-linked recessive form results from mutations in the gene *DKC1*, the product of which, dyskerin, is important for stabilisation of the RNA–protein complex known as telomerase.[9,10] Disruption of telomerase leads to accelerated telomere shortening, bone-marrow failure, and premature ageing. Autosomal dominant dyskeratosis congenita results from mutations of *TERC*, which encodes the RNA component of telomerase. Together with telomerase reverse transcriptase, TERC forms the core of the active telomerase complex; hence, *TERC* mutations also interfere with telomerase activity and lead to abnormally short telomeres.[11] Less than 5% of patients with suspected acquired aplastic anaemia have been found to have *TERC* mutations.[12,13]

Inherited amegakaryocytic thrombocytopenia is characterised by severe thrombocytopenia and absence of megakaryocytes at birth. Missense or nonsense mutations in *C-MPL* are present in most patients. A high proportion of these patients subsequently develop multilineage bone-marrow failure in the second decade of life.[14] Schwachman-Diamond syndrome is an autosomal recessive disorder characterised by pancreatic exocrine dysfunction, metaphyseal dysostosis, and bone-marrow failure.[15] As in Fanconi's anaemia, there is an increased risk of development of myelodysplasia or leukaemia at an early age. No causative genetic lesion has been identified, but a mutation in a gene (*SBDS*) located on chromosome 7 has been associated with this disease.[16]

Search strategy

We identified studies published before August, 2004, by searching the MEDLINE databases. The initial search term was "aplastic an[a]emia". We also searched reference lists from these reports and from earlier reviews. We did not restrict our search by language.

Disease and gene defect	Inheritance	Diagnostic assay	Comments
Fanconi's anaemia			
At least ten different genes cause phenotype	Autosomal recessive	Diepoxybutane, mitomycin	Increased rate of MDS/leukaemia and solid tumours owing to DNA-repair defect
Dyskeratosis congenita			
Unknown	Autosomal recessive	None	Classic triad: abnormal skin pigmentation, nail dystrophy, mucosal leucoplakia; predisposition to malignant disorders and pulmonary complications
DKC1	X-linked recessive	Sequence analysis of *DKC1*	
hTERC	Autosomal dominant	Sequence analysis of *hTERC* gene	
Amegakaryocytic thrombocytopenia			
C-MPL mutations	Autosomal recessive	Sequence analysis of *C-MPL* gene	Severe thrombocytopenia precedes bone-marrow failure
Schwachman-Diamond syndrome			
SBDS mutations in most patients	Autosomal recessive	None	Exocrine pancreatic deficiency and metaphyseal dysostosis; increased rate of MDS/leukaemia

MDS=myelodysplastic syndrome.

Table 1: **Characteristics and genetics of selected congenital bone-marrow-failure disorders**

Epidemiology

Acquired aplastic anaemia most commonly presents between the ages of 15 years and 25 years, but there is a second smaller peak in incidence after age 60 years. As in other autoimmune diseases, certain histocompatibility locus specificities, especially HLA DR2, are associated with an underlying predisposition to acquired aplastic anaemia.[17] Precise estimates of the incidence are confounded by imprecision in establishment of the diagnosis. The best estimates are from case-control studies. Such investigations have found an incidence of two cases per million inhabitants in Europe[18] and Israel;[19] the incidence seems to be two to three times higher in southeast Asia.[20,21] Although acquired aplastic anaemia has been causally associated with many agents, including drugs, benzene exposure, insecticides, and viruses, no aetiological agent can be identified in most cases.[22,23] A population-based case-control study of aplastic anaemia in Thailand found that drugs were the most commonly implicated cause, but they explained only 5% of newly diagnosed cases.[22]

An intriguing association between aplastic anaemia and hepatitis is found in 1% of new cases. Most cases of the syndrome of hepatitis and aplastic anaemia are seronegative for known hepatitis viruses (A to G). Severe pancytopenia typically begins 2–3 months after the serum concentrations of aminotransferases have peaked and responds to immunosuppression in many cases.[24,25] In addition, after orthotopic liver transplantation for seronegative hepatitis, up to 30% of patients develop aplastic anaemia.[26,27] Whether the hepatitis associated with aplastic anaemia is autoimmune or results from an undiscovered virus or toxin remains unclear.

Pathophysiology

Aplastic anaemia was originally thought to result from a direct toxic effect on haemopoietic stem cells that led to a decrease in their numbers. In the late 1960s, Mathé and colleagues were among the first to postulate an autoimmune basis for the disorder.[28] They transplanted bone marrow from partially mismatched donors to patients with aplastic anaemia, after administering antilymphocyte globulin for immunosuppression. Although the transplanted marrow did not engraft, there was autologous recovery of haemopoiesis in some patients. These findings suggested that patients with aplastic anaemia retained haemopoietic stem cells, the growth and differentiation of which were being suppressed by the immune system. An analysis by the International Bone Marrow Transplant Registry of bone-marrow transplants between identical twins for aplastic anaemia also suggested an autoimmune aetiology for most of the patients. Attempts to treat aplastic anaemia by simple transfusion of bone marrow from an identical twin failed to reconstitute haemopoiesis in about 70% of patients.[29] However, repetition of the procedure after a high-dose cyclophosphamide conditioning regimen able to eliminate autoimmunity was successful in most patients.[29,30]

The first laboratory evidence of autoimmunity in aplastic anaemia came from experiments showing that lymphocytes inhibited allogeneic and autologous haemopoietic colony formation in vitro.[31,32] Subsequently, cytotoxic T lymphocytes were found to mediate the destruction of haemopoietic stem cells in the disorder.[33,34] Effector T cells are more conspicuous in the bone marrow than in the peripheral blood of patients with aplastic anaemia,[35–37] and the cells produce interferon γ and tumour necrosis factor.[38,39] These cytokines are direct inhibitors of haemopoiesis and seem to upregulate Fas expression on CD34-positive cells.[40] Immortalised clones of T cells positive for CD4 and CD8 from patients with aplastic anaemia also secrete T-helper-1 cytokines and are directly toxic to autologous CD34-positive cells.[33,34] There is also evidence for a humoral autoimmune response in aplastic anaemia. Patients with aplastic anaemia have autoantibodies against kinectin, a 1300-aminoacid protein expressed on human haemopoietic cells and

liver, ovary, testis, and brain cells.[41] Studies of T-cell diversity by use of complementarity-determining-region (CDR3) spectratyping further implicated an autoimmune pathophysiology in aplastic anaemia. T cells from patients with the disorder showed limited heterogeneity of the T-cell-receptor β chain, which suggests oligoclonal T-cell expansion in response to a specific antigen.[34,42,43]

CD34-positive cells, and assayable haemopoietic progenitors, are greatly reduced in number in aplastic anaemia. However, although myeloid (granulocytic, erythroid, and megakaryocytic) deficiencies are universal in the disorder, immunological deficiencies are uncommon; lymphocyte counts are normal in most cases, as is B-cell and T-cell function.[44,45] Furthermore, complete recovery of normal haemopoiesis can occur with effective immunosuppressive therapy. Thus, haemopoietic stem cells seem to persist in many patients with aplastic anaemia. T cells from patients kill haemopoietic stem cells in a HLA-DR-restricted manner,[34,38] via Fas ligand.[40] Haemopoietic stem cells represent several classes of cells with varying capacity for long-term production of the different haemopoietic lineages.[46,47] The most primitive haemopoietic stem cells express little or no HLA DR[46,48] or Fas,[49,50] and the expression of both HLA DR and Fas increases as the stem cells mature. Thus, the primitive haemopoietic stem cells, which normally represent less than 10% of the total CD34-positive cells,[51] could be relatively imperceptible to the autoreactive T cells; instead, the more mature haemopoietic stem cells might be the main targets of the immune attack in aplastic anaemia (figure 1). The primitive haemopoietic stem cells eluding the autoimmune attack could allow the slow haemopoietic recovery that occurs in patients with aplastic anaemia after immunosuppressive therapy.

Clinical features and diagnosis

Aplastic anaemia can present abruptly (over days) or insidiously over weeks to months. Clinical manifestations are proportional to the peripheral-blood cytopenias and can include dyspnoea on exertion, fatigue, easy bruising, petechiae, epistaxis, gingival bleeding, heavy menses, headache, and fever. A complete blood count, leucocyte differential, reticulocyte count, and a bone-marrow aspirate and biopsy can establish the diagnosis. Peripheral-blood flow cytometry to rule out paroxysmal nocturnal haemoglobinuria[52,53] and bone-marrow karyotyping to help exclude hypoplastic myelodysplastic syndromes should be done for all patients. Patients younger than 40 years should be screened for Fanconi's anaemia by use of the clastogenic agents diepoxybutane or mitomycin, which test for the increased chromosomal breakage seen with this disorder.[5] A family history of cytopenias should raise suspicion of an inherited disorder even when no physical abnormalities are present.

A hypocellular bone marrow is required for the diagnosis of aplastic anaemia. Spicules from an aspirate

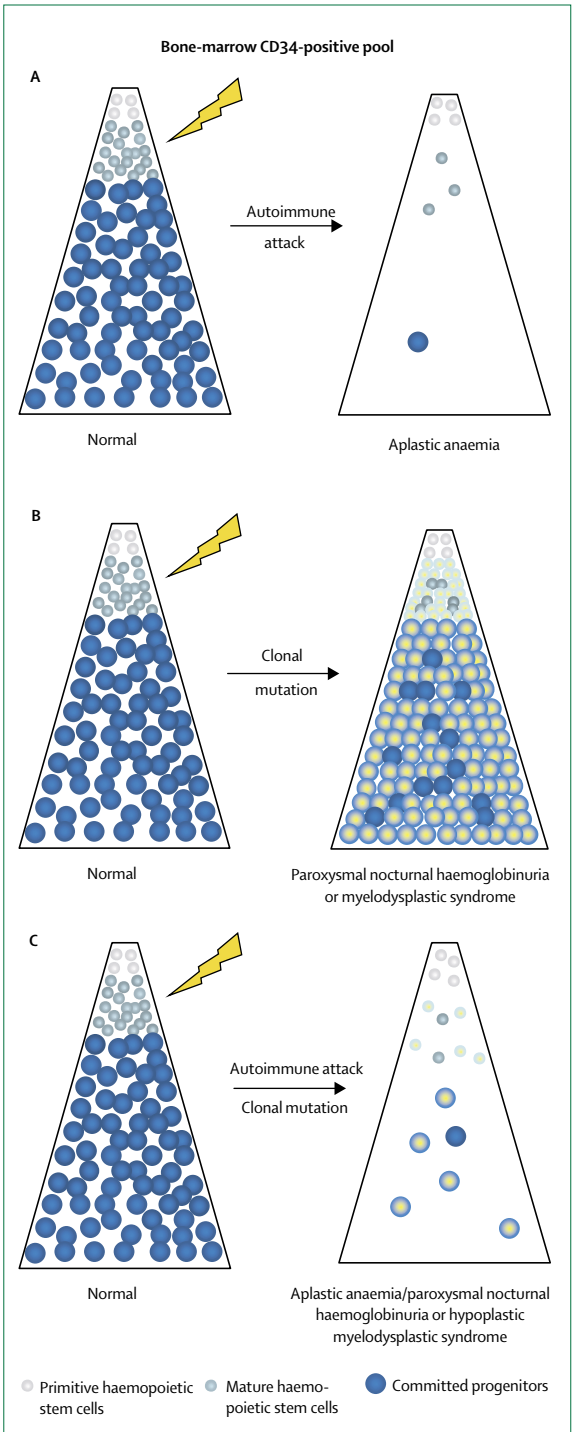

Figure 1: **Potential consequences of bone-marrow injury**
A: Injury exclusively generates an autoimmune reaction against haemopoietic stem cells or progenitors. B: Injury exclusively induces a genetic mutation in haemopoietic stem cells that results in clonal expansion. C: Injury leads to both an autoimmune reaction against, and clonal expansion of, haemopoietic stem cells. Depending on the rate of clonal growth, the clonality could occur simultaneously with the autoimmune attack (aplastic anaemia/paroxysmal nocturnal haemoglobinuria overlap or hypoplastic myelodysplastic syndrome) or subsequently (secondary clonal disease).

are surprisingly cellular in some patients despite overall marrow hypocellularity because most patients have residual pockets of continuing haemopoiesis. Thus, a 1–2 cm core biopsy is essential for assessment of cellularity. Mild dyserythropoiesis is not uncommon in aplastic anaemia, especially in patients with simultaneous small to moderate populations of paroxysmal-nocturnal-haemoglobinuria cells. However, the presence of a small proportion of myeloid blasts, or dysplastic features in the myeloid or megakaryocyte lineages, favours a diagnosis of a hypoplastic myelodysplastic syndrome. Distinction between aplastic anaemia and hypoplastic myelodysplastic syndromes can be challenging, especially in older patients among whom the latter syndromes are more common. The proportion of CD34-positive cells in the bone marrow can be helpful in some cases. CD34 is expressed on haemopoietic stem cells and progenitors and is fundemental to the pathophysiology of both disorders (figure 1). In myelodysplastic syndrome, clonal expansion emanates from a CD34-positive stem cell; in acquired aplastic anaemia, the CD34-positive stem cells are the target of an autoimmune attack. Accordingly, the proportion of CD34-positive cells is 0·3% or less in most patients with aplastic anaemia, whereas the proportion is normal (0·5–1·0 %) or higher than normal in hypoplastic myelodysplastic syndromes.

Classification of acquired aplastic anaemia

Like most autoimmune diseases, aplastic anaemia encompasses a wide range of disease activity from very mild to severe. The risk of morbidity and mortality from aplastic anaemia correlates better with the severity of the cytopenias than with bone-marrow cellularity. Thus, acquired aplastic anaemia is classified as non-severe, severe, or very severe on the basis of the degree of peripheral-blood pancytopenia. Bone-marrow cellularity of less than 25% and very low values of at least two of three haemopoietic lineages (neutrophil count $<0{\cdot}5\times10^9$/L, platelet count $<20\times10^9$/L, and absolute reticulocyte count $<60\times10^9$/L) defines the severe category. Very severe aplastic anaemia meets the same criteria except that the neutrophil count is below $0{\cdot}2\times10^9$/L. The non-severe category is characterised by a hypocellular bone marrow but the cytopenias do not meet the criteria for severe disease. The 2-year mortality rate with supportive care alone for patients with severe or very severe aplastic anaemia approaches 80%;[54] invasive fungal infections and overwhelming bacterial sepsis are the most common causes of death. Non-severe aplastic anaemia is seldom life-threatening, and in most cases no therapy is necessary.

Treatment

There is little evidence on the outlook for patients with non-severe aplastic anaemia. Although the disorder can progress, many patients remain stable for years, and some spontaneously improve even without specific treatment.[55] Treatment should be based on the degree of cytopenia, not the marrow cellularity. Patients with asymptomatic cytopenias probably need no treatment. Those with more severe cytopenias, such as symptomatic anaemia, might benefit from a trial of immunosuppressive therapy with antithymocyte globulin and ciclosporin. Whether treatment of non-severe aplastic anaemia affects survival is not clear.

There are three effective treatments for acquired severe aplastic anaemia: allogeneic bone-marrow transplantation; immunosuppressive therapy with antithymocyte globulin and ciclosporin; and high-dose cyclophosphamide without transplantation of bone marrow. These approaches differ substantially in their applicability and their short-term and long-term risks. Allogeneic bone-marrow transplantation eradicates the immune attack with high-dose cytotoxic therapy and provides a fresh source of stem cells; thus, it should be effective against all forms of bone-marrow failure. Antithymocyte globulin plus ciclosporin and high-dose cyclophosphamide primarily target an autoimmune attack, allowing endogenous stem cells to re-establish normal or near-normal haemopoiesis.

Bone-marrow transplantation

The treatment of choice at most centres for young patients with severe aplastic anaemia who have an HLA-matched sibling donor is bone-marrow transplantation (table 2). Survival after bone-marrow transplantation from a matched sibling has improved steadily since the 1970s, largely as a result of improved supportive care especially in prophylaxis against graft-versus-host disease (GVHD).[56] The probability that severe aplastic anaemia will be cured with bone-marrow transplantation is high, but less than 30% of patients have a matched sibling donor. Furthermore, late complications, such as chronic GVHD, occur in up to a third of patients, with many requiring long-term therapy

Study; year	Regimen	n	Median (range) age, years	Engraftment (%)	Survival (%)	Median follow-up, years	Acute GVHD (%)	Chronic GVHD (%)
FHCRC, Stanford, City of Hope, VAPSHCS; 1988–92[57]	High-dose cyclophosphamide, antithymocyte globulin	94	26 (2–59)	96	88	6	29	32
IBMTR; 1988–92[58]	Varied	471	20 (1–51)	84	66	3	19	32
EBMT; 1976–98[23]	Varied	1699	19 (1–69)	88	66	4	13	23

FHCRC=Fred Hutchinson Cancer Research Center; VAPSHCS=VA Puget Sound Health Care System; IBMTR=International Bone Marrow Transplant Registry; EBMT=European Group for Blood and Marrow Transplantation.

Table 2: **Representative results from large mature studies of bone-marrow transplantation for severe aplastic anaemia**

Study; year	Regimen	n	Median (range) age, years	Response (%)	Survival (%)	Median follow-up, years	Relapse (%)	MDS or leukaemia (%)
NIH; 1991–98[65]	Antithymocyte globulin and ciclosporin	122	35	61	55	7·2	35	10
GAASG; 1986–89[66]	Antithymocyte globulin and ciclosporin	43	32 (7–80)	70	58	7	45	8
GITMO/EBMT; 1991–99[67]	Antilymphocyte globulin and ciclosporin, filgrastim	100	16 (1–72)	77	87	3	12	3

MDS=myelodysplastic syndrome; NIH=National Institutes of Health; GAASG=German Aplastic Anemia Study Group; GITMO=Gruppo Italiano Trapianti di Midollo Osseo.

Table 3: **Representative results of large studies of immunosuppressive therapy for severe aplastic anaemia**

for the GVHD.[57] A major advantage of bone-marrow transplantation is that it greatly reduces the risk of relapse and the outgrowth of late clonal disorders such as myelodysplastic syndrome and paroxysmal nocturnal haemoglobinuria.[59]

The age of the patient and the type of allograft (HLA-matched sibling, unrelated, or mismatched donor) are the most important factors influencing outcome. In patients younger than 30 years, cure rates after transplantation of bone marrow from an HLA-matched sibling are between 70% and 90%.[57,60,61] However, the risk of GVHD increases steadily with age, so survival is poorer in older patients. Transplant-related mortality approaches 50% in patients older than 40 years.[61,62] Bone-marrow transplantation from unrelated or mismatched donors is generally reserved for patients who do not respond to antithymocyte globulin and ciclosporin. Transplant-related mortality and the risk of GVHD are roughly two times higher with unrelated donors than with matched sibling donors.[23] A retrospective analysis of 141 patients with severe aplastic anaemia who underwent transplantation from unrelated donors between 1988 and 1995 revealed overall survival of 36%. Somewhat better results have been reported in single-institution studies. Results with unrelated transplants are best in patients younger than 21 years with disease duration of less than a year.[63,64]

Immunosuppressive therapy

Immunosuppressive therapy with antithymocyte globulin and ciclosporin is used in patients who are not candidates for bone-marrow transplantation because they are older or lack a matched sibling donor. Response rates to antithymocyte globulin and ciclosporin range between 60% and 80% (table 3), with 5-year survival similar to those after bone-marrow transplantation.[68] However, in contrast to bone-marrow transplantation, in most of these patients the disease is not cured. Most patients do not achieve normal blood counts,[66,69] and many relapse, become dependent on ciclosporin, or develop secondary clonal disease such as paroxysmal nocturnal haemoglobinuria or myelodysplastic syndrome.[65,67] The European Group for Blood and Marrow Transplantation (EBMT) Severe Aplastic Anaemia Working Party did a retrospective analysis of 468 patients treated with immunosuppressive therapy between 1975 and 1986.[70] Those who survived longer than 2 years were classified as long-term survivors. The actuarial mortality rate for these 223 long-term survivors was 22% at 8 years; and the actuarial risks of paroxysmal nocturnal haemoglobinuria and myelodysplastic syndrome were 13% and 15%, respectively, at 7 years. Bacigalupo and colleagues[67] reported a response rate of 77% and overall survival of 87% in 100 patients (median age, 16 years) treated with antithymocyte globulin and ciclosporin, prednisolone, and filgrastim. The risk of relapse was 9%, cytogenetic abnormalities developed in 11%, but the actuarial probability of stopping ciclosporin therapy was only 38% at 5 years. The US National Institutes of Health treated 122 patients (median age, 35 years) with the combination of antithymocyte globulin and ciclosporin plus methylprednisolone and followed them up over a period of 8 years.[65] The response rate was 58%, and actuarial survival at 7 years was 55%; 13% of patients died within 3 months of treatment, mostly from fungal infections. The relapse rate for responders was 40%, and 13 patients developed myelodysplastic syndrome.

High-dose cyclophosphamide without bone-marrow transplantation

Cyclophosphamide in high doses (commonly in conjunction with antithymocyte globulin) remains the most commonly used conditioning regimen before bone-marrow transplantation for aplastic anaemia.[57,71] Complete reconstitution of autologous haemopoiesis occurs in 10–20% of patients undergoing allogeneic bone-marrow transplantation for aplastic anaemia, largely owing to cyclophosphamide's unique pharmacology.[72–74] Most of these patients have maintained long-term remissions despite autologous reconstitution.

Cyclophosphamide is a prodrug and is converted to 4-hydroxycyclophosphamide and its tautomer aldophosphamide by the hepatic cytochrome P450 system (figure 2). These unstable bloodstream transport precursors diffuse into cells. In cells with low concentrations of aldehyde dehydrogenase (ALDH; sensitive cells), aldophosphamide is converted into phosphoramide mustard and acrolein through simple intracellular decomposition. The active alkylating compound, phosphoramide mustard, produces the interstrand and intrastrand DNA cross-links that are the cytotoxic result of the drug; acrolein is excreted through the kidneys and in high doses leads to uroepithelial

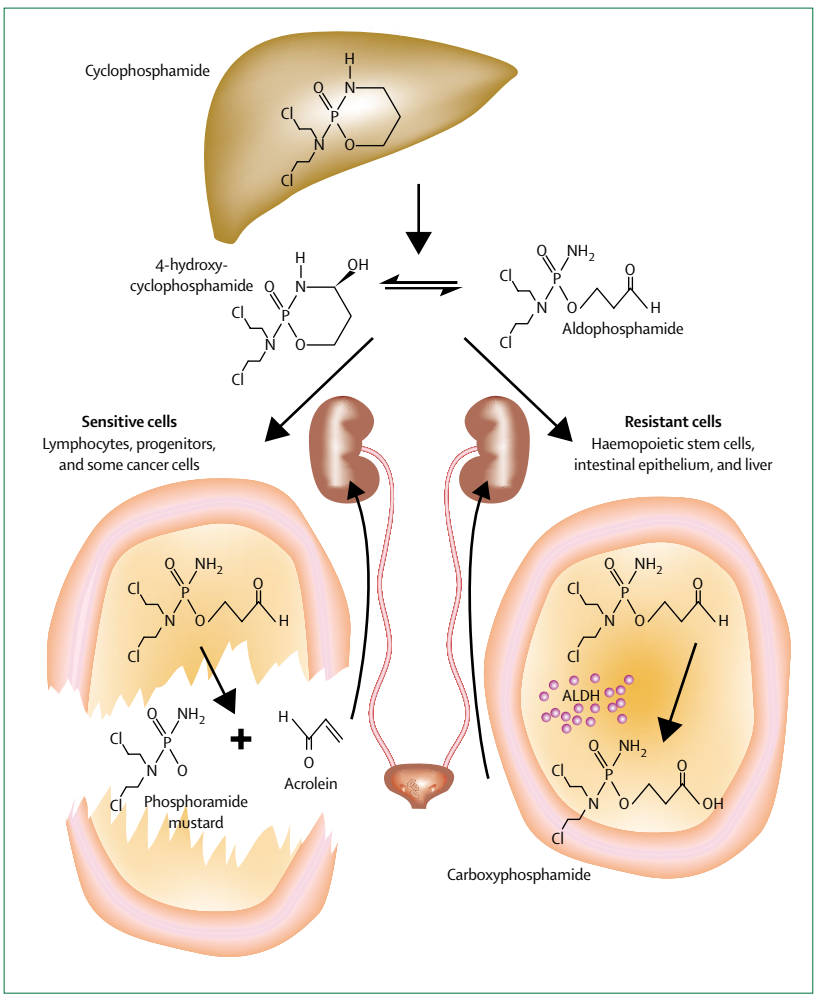

Figure 2: **Cyclophosphamide metabolic pathway**

injury, haemorrhagic cystitis. In cells with high concentrations of ALDH (resistant cells), aldophosphamide is converted to carboxyphosphamide, an inert compound that is excreted by the kidneys. ALDH1A1, previously called class I or cytosolic ALDH, ALDH1, or retinaldehyde dehydrogenase 1, seems to be the form most active in this cyclophosphamide detoxification process.[75] ALDH1A1 also has an important role in ethanol metabolism, but its major function is the biosynthesis of retinoic acid from vitamin A (retinol).[75] After oxidisation of retinol to retinaldehyde by alcohol dehydrogenase, ALDH1A1 oxidises retinaldehyde to retinoic acid, which is essential for cellular growth and differentiation. Cells with high proliferative potential, such as haemopoietic stem cells, have high expression of ALDH1A1 because of their requirement for retinoic acid; they are therefore resistant to cyclophosphamide.[76–78] By contrast, lymphocytes have low expression of ALDH and are rapidly killed by high doses of cyclophosphamide. This drug is therefore highly immunosuppressive, but not myeloablative, allowing endogenous haemopoietic stem cells to reconstitute haemopoiesis.

High-dose cyclophosphamide without bone-marrow transplantation has been successfully used to treat patients with aplastic anaemia who lacked a suitable donor.[79–82] In a pilot study, seven of ten patients achieved durable complete remissions. No relapse or clonal evolution was observed during median follow-up of 10·8 years (range 7·3–17·8). Investigators at the National Institutes of Health initiated a randomised trial of high-dose cyclophosphamide plus ciclosporin versus antithymocyte globulin and ciclosporin.[83] The trial closed after enrolling 31 patients (17% of the planned accrual) because the frequency of early toxic effects was higher in the cyclophosphamide plus ciclosporin group. However, no stopping rules were met, and there were no statistical differences in response rate, survival, or secondary clonal disorders. Subsequently, we and colleagues[84] gave high-dose cyclophosphamide to 19 patients with severe aplastic anaemia; 16 were alive at 24 months, with 14 patients achieving complete or partial remission. There were no relapses or secondary clonal disorders. The median time to achieve a neutrophil count of $0{\cdot}5\times10^9$/L was 49 days, and the median time to last platelet transfusion was 129 days. That study showed that addition of ciclosporin to the high-dose cyclophosphamide regimen is not necessary because it probably increases toxicity without improving efficacy. High-dose cyclophosphamide has been shown to be successful in patients who did not respond to antithymocyte globulin and ciclosporin. Nine of 17 patients who did not respond or relapsed after one or more rounds of immunosuppressive therapy achieved durable remissions after high-dose cyclophosphamide. Larger series and randomised trials will be necessary to define the role of high-dose cyclophosphamide in patients with severe aplastic anaemia; until then, this promising therapy should be considered investigational.

Supportive care

Transfusions

Patients with symptomatic anaemia or thrombocytopenia associated with wet purpura or bleeding need immediate transfusions. All transfusions in patients with suspected aplastic anaemia should be irradiated to prevent transfusion-associated GVHD. If the patient is a potential candidate for bone-marrow transplantation and is negative or of unknown status for cytomegalovirus, transmission of this virus should be avoided by either leucoreduction or the use of cytomegalovirus-negative products. Blood donation from family members should be avoided to prevent alloimmunisation that could also complicate future bone-marrow transplantation. After stabilisation of the patient, blood products should be used judiciously to prevent cardiopulmonary compromise and to reduce the risk of haemorrhage; a target platelet count of 10×10^9/L will be sufficient for

most patients, although some will tolerate even lower target platelet counts without bleeding or petechiae.

Growth factors

Deficiency of haemopoietic growth factors (such as erythropoietin, granulocyte-colony-stimulating factor, thrombopoietin, and granulocyte-monocyte-colony-stimulating factor) is not the cause of the bone-marrow failure in aplastic anaemia; concentrations of haemopoietic growth factors are very high in patients with the disorder, in a compensatory attempt to increase blood production. Hence, these factors should not be used instead of definitive therapy. Haemopoietic growth factors are commonly used after immunosuppressive therapy or high-dose cyclophosphamide to accelerate haemopoietic recovery, but their use has not been shown to improve survival.

Antibiotics

Overwhelming sepsis caused by bacteria or fungi (especially aspergillus) is the most frequent cause of death from aplastic anaemia. In most circumstances, prophylactic antibiotics are unnecessary. However, for patients with absolute neutrophil counts that are consistently lower than $0{\cdot}2\times10^9$/L, oral prophylaxis with a quinolone and a triazole antifungal drug is reasonable. Patients with febrile neutropenia should be treated promptly with broad-range antibiotics; in those with persistent fever after the initiation of antibacterial drugs, agents against aspergillus should be added. Prophylaxis for *Pneumocystis carinii* pneumonia should be given to all patients for at least 6 months after immunosuppressive therapy, bone-marrow transplantation, or high-dose cyclophosphamide therapy.

Aplastic anaemia and clonality

One of the most controversial and potentially biologically important issues surrounding aplastic anaemia is the high incidence of concomitant clonal haemopoiesis (ie, myelodysplastic syndrome and paroxysmal nocturnal haemoglobinuria). Even before the widespread use of immunosuppressive therapy, 5% of patients with aplastic anaemia showed progression to clonal haemopoiesis. This evidence suggests that the increase in myelodysplastic syndrome and paroxysmal nocturnal haemoglobinuria after immunosuppressive therapy is not a direct consequence of the treatment. Instead, the longer survival after immunosuppressive therapy probably allows time for these underlying clones to develop and expand.[85,86]

Myelodysplastic syndrome is a clonal haemopoietic-stem-cell disorder that produces multilineage haematological cytopenias. It is associated with heterogeneous karyotypic abnormalities, commonly involving chromosome 5, 7, or 8. Up to 15% of children and adults with acquired aplastic anaemia develop myelodysplastic syndrome after immunosuppressive therapy; monosomy 7 is the most common chromosomal abnormality.[65,87] Paroxysmal nocturnal haemoglobinuria results from the expansion of an abnormal haemopoietic stem cell that has a somatic mutation of the X-linked gene, *PIG-A*.[88,89] The product of this gene is needed for biosynthesis of glycosyl-phosphatidylinositol anchors; thus, paroxysmal-nocturnal-haemoglobinuria cells are deficient in all glycosylphosphatidylinositol-anchored proteins. Several of these proteins (CD59 and CD55) protect cells from complement-mediated destruction, and their absence explains the haemolytic anaemia associated with paroxysmal nocturnal haemoglobinuria.

The leading hypothesis to explain the close relation between paroxysmal nocturnal haemoglobinuria and aplastic anaemia, and the mechanisms by which the paroxysmal-nocturnal-haemoglobinuria clone achieves dominance, involves a two-step model. It proposes that haemopoietic stem cells randomly and spontaneously acquire *PIG-A* mutations at a very low frequency. Indeed, *PIG-A*-mutant granulocytes are found at low frequency in most healthy people.[90] However, later work has suggested that haemopoietic stem cells in healthy people do not have *PIG-A* mutations, and thus question the relevance of these mutations to the pathogenesis of paroxysmal nocturnal haemoglobinuria.[91] The *PIG-A* mutations in healthy people seem to arise in differentiated progenitor cells that lack self-renewal capacity, probably as a function of normal differentiation. DNA repair is progressively attenuated during the process of cellular differentiation,[92] possibly leading to differentiation-dependent spontaneous mutations.[93] Step two in the model proposes that the immunological attack that targets haemopoietic stem cells in aplastic anaemia spares paroxysmal-nocturnal-haemoglobinuria cells, ostensibly because they lack glycosylphosphatidylinositol-anchored proteins.[94,95] However, there is no direct evidence to support a glycosylphosphatidylinositol-anchored protein being the target of the immune attack in aplastic anaemia. Importantly, this two-step model does not explain the similarly high incidence of myelodysplastic syndrome in patients with aplastic anaemia.[59,96]

Another hypothesis that could explain the predisposition of patients with aplastic anaemia to both paroxysmal nocturnal haemoglobinuria and myelodysplastic syndrome has been proposed. The secondary disorders in the setting of aplastic anaemia could be analogous to the "field cancerisation effect" described in aerodigestive and other solid tumours to explain second primary tumours in the affected tissue.[97,98] Thus, a single insult to the marrow, such as general toxic damage or a genetic predisposition, could bring about different forms of marrow disorders; these disorders might occur alone, simultaneously, or sequentially (figure 1).[99,100] In aplastic anaemia, the bone-marrow injury might primarily trigger an autoimmune

attack on the haemopoietic stem-cell or progenitor compartment, perhaps by exposing cryptic epitopes, through molecular mimicry, or by sending a "danger" signal (figure 1, A). In primary paroxysmal nocturnal haemoglobinuria or myelodysplastic syndrome, the injury might first produce a genetic mutation that leads to clonal dominance of the affected clone (figure 1, B).[101–103] In other patients, bone-marrow injury might induce both an autoimmune haemopoietic attack and a clonal genetic mutation that present either simultaneously or sequentially (figure 1, C). Examples of simultaneous presentation are the overlap between aplastic anaemia and paroxysmal nocturnal haemoglobinuria and hypoplastic myelodysplastic syndrome. Alternatively, the expansion of an inconspicuous population of damaged haemopoietic stem cells might subsequently manifest as myelodysplastic syndrome or paroxysmal nocturnal haemoglobinuria after sufficient lag time provided by the longer survival afforded patients with aplastic anaemia by antithymocyte globulin and ciclosporin.

Conclusion

Although aplastic anaemia has not generally been included in the inventory of autoimmune diseases, it certainly should be because in most of these patients there is an autoimmune basis for the disease. Moreover, both the range of disease activity, from moderate to severe and life-threatening, and the type of treatment correspond to those for other autoimmune disorders. Emerging data on the effectors and targets of the immune attack are also beginning to shed new light on the pathophysiology of aplastic anaemia and to suggest new therapeutic approaches. However, even with meticulous investigation to identify those patients with inherited forms of bone-marrow failure such as Fanconi's anaemia and dyskeratosis congenita, or clonal haemopoietic-stem-cell disorders such as hypoplastic myelodysplastic syndromes, a substantial minority of cases of apparent acquired aplastic anaemia result from non-autoimmune causes. Inherited defects in telomerase function and telomere maintenance contribute to the pathogenesis of the bone-marrow failure in a small subset of patients with aplastic anaemia, even when no familial tendency is present.[104] The exact proportion of apparent acquired aplastic anaemia that does not have autoimmune basis is unknown, but it is probably less than 30%.[29] As the ability to assess autoimmunity, hypoplastic myelodysplastic syndromes, and inherited genetic abnormalities improves, the number of non-autoimmune cases classified as acquired aplastic anaemia should decrease.

Contributions

Robert A Brodsky and Richard J Jones contributed equally in the drafting and revisions of this Seminar. Robert A Brodsky is a Clinical Research Scholar of the Leukemia Lymphoma Society. There was no funding source for this Seminar.

Conflict of interest statement

We declare that we have no conflicts of interest.

Acknowledgments

We thank Jerry Spivak for helpful discussions and Kariann Glynn for her help in preparing the paper.

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