

Review in translational hematology

Current concepts in the pathophysiology and treatment of aplastic anemia

Neal S. Young, Rodrigo T. Calado, and Phillip Scheinberg

Aplastic anemia, an unusual hematologic disease, is the paradigm of the human bone marrow failure syndromes. Almost universally fatal just a few decades ago, aplastic anemia can now be cured or ameliorated by stem-cell transplantation or immunosuppressive drug therapy. The pathophysiology is immune mediated in most cases, with activated type 1 cytotoxic T cells implicated. The molecular basis of the aberrant immune response and deficiencies in hematopoietic cells is now being defined genetically; examples

are telomere repair gene mutations in the target cells and dysregulated T-cell activation pathways. Immunosuppression with antithymocyte globulins and cyclosporine is effective at restoring blood-cell production in the majority of patients, but relapse and especially evolution of clonal hematologic diseases remain problematic. Allogeneic stem-cell transplant from histocompatible sibling donors is curative in the great majority of young patients with severe aplastic anemia; the major challenges are extending the ben-

efits of transplantation to patients who are older or who lack family donors. Recent results with alternative sources of stem cells and a variety of conditioning regimens to achieve their engraftment have been promising, with survival in small pediatric case series rivaling conventional transplantation results. (Blood. 2006;108:2509-2519)

© 2006 by The American Society of Hematology

Introduction

More than 25 years have passed since our first, highly speculative review of aplastic anemia; in that fortunately obscure publication, pathophysiology was addressed tentatively and immunosuppressive therapies hardly at all. The article did reflect both the dismal prospects for patients with the severe form of marrow failure and the formidable practical difficulties of experimentation in a rare disorder in which the cells of interest had disappeared. Aplastic anemia was considered heterogenous in origin and virtually impossible to study systematically. At the bedside, the clinical emphasis was the identification of a putative causal factor—exposure to benzene or a culpable pharmaceutical—to allow classification in an otherwise doomed patient. As progenitor assays were developed, diverse factors could be held theoretically responsible for failure to form colonies in tissue culture, ranging from quantitative and qualitative defects in stem cells and blocks in differentiation to a lack of stroma support or inadequate cytokine production, or the effects of a chemical poison.

In the intervening decades, our understanding of aplastic anemia has cohered around a unified immune mechanism of hematopoietic-cell destruction, which was inferred from but also has informed effective immunosuppressive therapies for the disease (Figure 1). Technical advances in cell biology, flow cytometry, molecular biology, and immunology have provided methods to measure numbers and function of very limited numbers of cells. As a result, we have a more unified and rational view of aplastic anemia's pathophysiology; the disease is understood in its relation to other related marrow failure syndromes; and in many important respects an unusual blood syndrome can model more common autoimmune diseases of other organ systems (Figure 2). Particularly satisfying is that aplastic anemia is now amenable to cure or

amelioration in most patients, based both on high-quality clinical trials and mechanistic insights from the experimental laboratory.

Our intention in this circumscribed review is to emphasize the most current aspects of aplastic anemia. As a complement, the reader is referred to monographs, textbook chapters, and other recent reviews.¹⁻⁶

Etiologies

Clinical associations

Since Ehrlich's description of the first case of aplastic anemia in a pregnant woman,¹²⁶ precipitating factors have been sought from the individual patient's history. An enormous literature, dating from the beginning of the 20th century, described chemical- and drug-induced disease, stimulated by observations of the effects of benzene on blood counts, of dipyrone's association with agranulocytosis, and a seeming epidemic of aplastic anemia after the introduction of chloramphenicol.

These associations are worth reassessment in the context of the immune hypothesis of marrow failure. Pregnancy appears a real association, as deduced more from the documented improvement of blood counts with its termination than from formal epidemiologic study.⁷ The unusual syndrome of eosinophilic fasciitis also is strongly linked to aplastic anemia. Five to 10% of cases of aplastic anemia follow an episode of seronegative hepatitis, in which immune activation is inferred from the pattern of T-cell activation, cytokine production, and HLA association.⁸ Despite intensive efforts, including sophisticated molecular and immunologic approaches and animal inoculations, an infectious agent has not been

From the Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health (NIH), Bethesda, MD.

Submitted March 22, 2006; accepted May 30, 2006. Prepublished online as *Blood* First Edition Paper, June 15, 2006; DOI 10.1182/blood-2006-03-010777.

Supported by NIH Intramural Research program.

N.S.Y. wrote "Introduction" and "Etiologies" as well as contributed to "Pathophysiology" and "Treatment"; R.T.C. wrote "Pathophysiology"; and P.S. wrote "Treatment." All authors contributed to the final paper.

Reprints: Neal S. Young, 10 Center Dr, Bldg 10/CRC, Rm 3E-5140, Bethesda, MD 20892-1202; e-mail: youngns@mail.nih.gov.

© 2006 by The American Society of Hematology

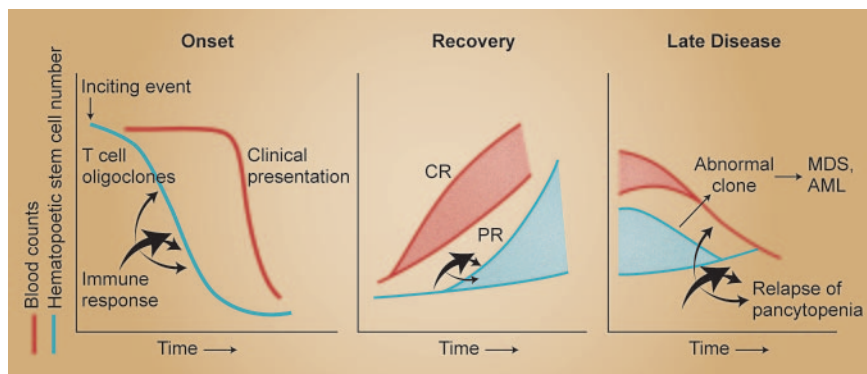


Figure 1. Pathophysiology of acquired aplastic anemia. The figure stresses the crucial and related roles of the hematopoietic stem-cell compartment as a target for the immune response. An inciting event, such as a virus or medical drug, provokes an aberrant immune response, triggering oligoclonal expansion of cytotoxic T cells that destroy hematopoietic stem cells (left panel, Onset). Bone marrow transplantation or immunosuppressive therapy leads to complete response (CR) or partial response (PR) by eradicating or suppressing pathogenic T-cell clones (middle panel, Recovery). Relapse occurs with recurrence of the immune response, and the immunologically stressed and depleted stem-cell compartment also allows selection of abnormal hematopoietic clones that manifest as paroxysmal nocturnal hemoglobinuria, myelodysplasia (MDS), and occasionally acute myelogenous leukemia (AML) (right panel, Late Disease).

identified. Benzene, or more correctly its metabolites, is a marrow toxin in animals and humans, but in the West benzene exposure as an etiology of aplastic anemia is now rare. Ancient case reports and series leave doubt as to whether marrow failure in benzene workers was not often myelodysplasia rather than aplastic anemia. Additionally, benzene also has effects on immune function.⁹

Of greatest practical import is the relationship of medical drug use to aplastic anemia—unpredictable marrow failure in this setting is devastating to the patient and physician and has serious legal ramifications for pharmaceutical drug development.¹⁰ The study of idiosyncratic drug reactions, by definition extremely rare, is difficult. That genetic differences in drug metabolism, especially in detoxification of reactive intermediate compounds, underlie susceptibility is best supported by one study of a single individual exposed to carbamazepine, published more than 20 years ago.¹¹ Overrepresentation of deletions in the drug-metabolizing glutathione-S-transferase genes (*GSTM1*, *GSTT1*, which would increase concentrations of toxic drug intermediates) has been observed in some series.^{12,13} Astonishingly, no satisfactory mechanism has been developed for the most notorious pharmaceutical, chloramphenicol, or for other heavily inculpated agents such as penicillamine or gold. Many drugs on “black lists” also more commonly cause mild marrow suppression, and possibly regular but only modest destruction of marrow cells is a prerequisite for a much more infrequent immune response to an exposed neoantigen. A parallel mechanism is supported by the clinical observation of

little difference between patients with idiopathic aplastic anemia and those with an assumed drug etiology, in demographics, response to therapy, or survival.¹⁴ In contrast, very few chemotherapeutic agents, despite being designed as cell poisons and administered in milligram or gram quantities, directly result in irreversible marrow destruction without obvious effects on other organs. Claims of permanent aplastic anemia after idiosyncratic exposure to minuscule quantities of chloramphenicol, for example (as in ophthalmic solutions), more likely reflect observation and reporting biases than a mechanism of extreme sensitivity to a hidden metabolite.

Epidemiology

Two more reliable approaches to identifying etiology are epidemiology and laboratory identification of antigens. Unfortunately, neither has yielded conclusive results. Two large, controlled, population-based studies have been conducted, the International Aplastic Anemia and Agranulocytosis Study in Europe and Israel in the 1980s¹⁵ and the recently completed Thai NHLBI Aplastic Anemia Study in Bangkok and a northeast rural region.¹⁶ The incidence of aplastic anemia in the West is 2/million and about 2- to 3-fold higher in Asia. Benzene and pesticides, while significantly associated, accounted for only a small number of cases in both studies, and medical drugs have a negligible role in Asia. In rural Thailand, exposure to nonbottled water, as well as to certain animals (ducks and geese), to animal fertilizer, and also to pesticides, suggested an infectious etiology.

Autoantigens

A few putative antigens have been teased from screening antibodies in patients’ sera against a peptide library (by expression of genes in fetal liver or leukemic-cell lines). Kinectin, a widely expressed protein, bound to antibodies from about 40% of aplastic patients.¹⁷ Another antigen that bound to antibodies, in a smaller minority of marrow failure patients, was diazepam-binding related protein-1, an enzyme essential in the oxidation of unsaturated fatty acids and broadly distributed in tissues.¹⁸ The relevance of these autoantibodies to a cellular pathophysiology of aplastic anemia is unclear. For kinectin, reactive cytotoxic T cells could be generated in vitro and inhibited human hematopoietic colony formation, but antikinctin T cells were not found in patients.¹⁷ For diazepam-binding related protein-1, a putative T-cell epitope derived from one patient, and T-cell precursors with peptide-binding activity were present in 2 cases.

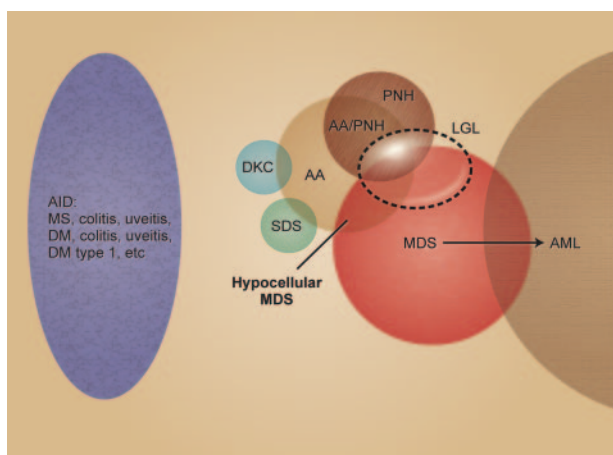


Figure 2. Venn diagram of the clinical and pathophysiological relationships among the bone marrow failure syndromes, leukemia, and autoimmune diseases. Overlapping circles indicate difficulties in diagnostic discrimination and shared underlying mechanisms.

Pathophysiology

In most cases, aplastic anemia is an immune-mediated disease. Cellular and molecular pathways have been mapped in some detail for both effector (T lymphocyte) and target (hematopoietic stem and progenitor) cells (Figure 3). Exposure to specific environmental precipitants, diverse host genetic risk factors, and individual differences in the characteristics of the immune response likely account for the disease's infrequency, variations in its clinical behavior, and patterns of responsiveness to treatment.

Immune-mediated T-cell destruction of marrow

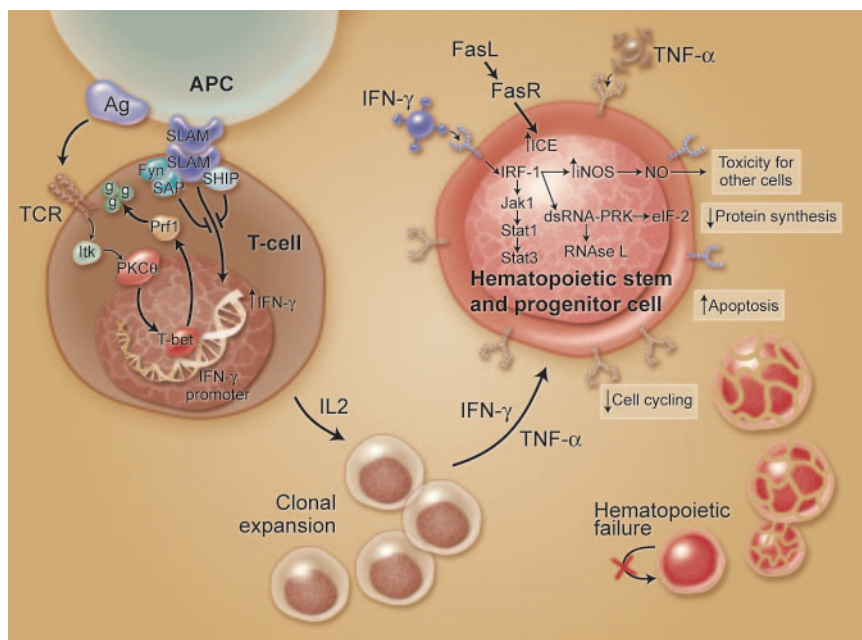
An immune mechanism was inferred decades ago from the recovery of hematopoiesis in patients who failed to engraft after stem-cell transplantation, when renewal of autologous blood-cell production was credited to the conditioning regimen. Also suggestive was that the majority of syngeneic transplantations in which bone marrow was infused without conditioning failed.¹⁹ The responsiveness of aplastic anemia to immunosuppressive therapies remains the best evidence of an underlying immune pathophysiology: the majority of patients show hematologic improvement after only transient T-cell depletion by antithymocyte globulins (ATGs); relapse also usually responds to ATG; and dependence of adequate blood counts on administration of very low doses of cyclosporine is not infrequent. As immunosuppression has intensified, from early attempts with corticosteroids to aggressive strategies such as high-dose cyclophosphamide, and the proportion of responders has risen, the willingness to ascribe an immunologic mechanism also increased. Indeed, little distinguishes responders to immunologic therapy from refractory patients (other than age, as children show higher rates of recovery and survival). A nonimmune pathophysiology has been inferred from a failure to respond to immunosuppression, but refractoriness to therapy is also consistent with very severe stem-cell depletion, a "spent" immune response, or immunologic mechanisms not susceptible to current therapies.

In early laboratory experiments, removal of lymphocytes from aplastic bone marrows improved colony numbers in tissue

culture, and their addition to normal marrow inhibited hematopoiesis in vitro (reviewed in Young²⁰). The effector cells were identified by immunophenotyping as activated cytotoxic T cells expressing Th1 cytokines, especially γ -interferon. CD8 cells containing intracellular interferon may now be measured directly in the circulation,²¹ and oligoclonal expansion of CD8⁺ CD28⁻ cells, defined by (1) flow cytometric analysis for T-cell receptor (TCR) V β subfamilies; (2) spectratyping to detect skewing of CDR3 length; and (3) sequencing of the CDR3 region to establish a molecular clonotype.²² In general, patients at presentation demonstrate oligoclonal expansions of a few V β subfamilies, which diminish or disappear with successful therapy; original clones re-emerge with relapse, sometimes accompanied by new clones, consistent with spreading of the immune response. Very occasionally, a large clone persists in remission, perhaps evidence of T-cell tolerance.

The impact of T-cell attack on marrow can be modeled in vitro and in vivo. γ -Interferon (and tumor necrosis factor- α) in increasing doses reduce numbers of human hematopoietic progenitors assayed in vitro; the cytokines efficiently induce apoptosis in CD34 target cells, at least partially through the Fas-dependent pathway of cell death.²³ In long-term culture of human bone marrow, in which stromal cells were engineered to constitutively express γ -interferon, the output of long-term culture-initiating cells (LTCI-ICs) was markedly diminished, despite low concentrations of the cytokine in the media, consistent with local amplification of toxicity in the marrow milieu.²⁴ Immune-mediated marrow failure has been modeled in the mouse: infusion of parental lymph node cells into F1 hybrid donors caused pancytopenia, profound marrow aplasia, and death.²⁵ Not only a murine version of ATG and cyclosporine but also monoclonal antibodies to γ -interferon and tumor necrosis factor abrogated hematologic disease, rescuing animals. A powerful "innocent bystander" effect, in which activated cytotoxic T cells kill genetically identical targets, was present in secondary transplantation experiments.²⁶ In a minor histocompatibility antigen-discordant model, marrow destruction resulted from activity of an expanded H60 antigen-specific T-cell clone.²⁷

Figure 3. Immune destruction of hematopoiesis. Antigens are presented to T lymphocytes by antigen-presenting cells (APCs), which trigger T cells to activate and proliferate. T-bet, a transcription factor, binds to the interferon- γ (IFN- γ) promoter region and induces gene expression. SAP binds to Fyn and modulates SLAM activity on IFN- γ expression, diminishing gene transcription. Patients with aplastic anemia show constitutive T-bet expression and low SAP levels. IFN- γ and TNF- α up-regulate other T cells' cellular receptors and also the Fas receptor. Increased production of interleukin-2 leads to polyclonal expansion of T cells. Activation of Fas receptor by the Fas ligand leads to apoptosis of target cells. Some effects of IFN- γ are mediated through interferon regulatory factor 1 (IRF-1), which inhibits the transcription of cellular genes and entry into the cell cycle. IFN- γ is a potent inducer of many cellular genes, including inducible nitric oxide synthase (NOS), and production of the toxic gas nitric oxide (NO) may further diffuse toxic effects. These events ultimately lead to reduced cell cycling and cell death by apoptosis.



Why T cells are activated in aplastic anemia is unclear. HLA-DR2 is overrepresented among patients, suggesting a role for antigen recognition, and its presence is predictive of a better response to cyclosporine.^{28,29} Polymorphisms in cytokine genes, associated with an increased immune response, also are more prevalent: a nucleotide polymorphism in the tumor necrosis factor- α (*TNF2*) promoter at -308,^{30,31} homozygosity for a variable number of dinucleotide repeats in the gene encoding γ -interferon,³² and polymorphisms in the interleukin 6 gene.³³ Constitutive expression of T-bet, a transcriptional regulator that is critical to Th1 polarization, occurs in a majority of aplastic anemia patients.³⁴ Mutations in *PRF1*, the gene for perforin, are responsible for some cases of familial hemophagocytosis; mutations in *SAP*, a gene encoding for a small modulator protein that inhibits γ -interferon production, underlie X-linked lymphoproliferation, a fatal illness associated with an aberrant immune response to herpesviruses and aplastic anemia. Perforin is overexpressed in aplastic marrow.³⁵ We have detected heterozygous mutations in *PRF1* in 5 adults with severe aplasia and hemophagocytosis of the marrow, and *SAP* protein levels are markedly diminished in a majority of acquired aplastic anemia cases (E. Solomou, unpublished data, June 2006). These alterations in nucleotide sequence and in gene regulation suggest a genetic basis for aberrant T-cell activation in bone marrow failure. Genome-wide transcriptional analysis of T cells from aplastic anemia patients has implicated components of innate immunity in aplastic anemia, including Toll-like receptors and natural killer cells,³⁶ for which there is some preliminary experimental support.^{37,38}

Hematopoiesis

Immune attack leads to marrow failure. "Anhematopoiesis" was inferred from the empty appearance of the marrow at autopsy by the earliest observers of the disease. The pallor of the modern biopsy core or empty spicules of an aspirate, few or no CD34 cells on flow cytometry, and minimal numbers of colonies derived from committed progenitors in semisolid media all reflect the severe reduction in hematopoietic cells that defines the disease. Stem-cell "surrogate"—really correlative—assays, LTC-ICs,³⁹ or cobblestone-forming cells,⁴⁰ which measure a primitive infrequent and quiescent multipotential progenitor cell, also show marked deficiency, and from the product of the low percentage of marrow cellularity and the scant numbers of LTC-ICs per mononuclear cell, suggest that only a small percentage of residual early hematopoietic cells remains in severely affected patients at presentation. Qualitative features of these few cells, as measured, for example, by poor colony formation per CD34 cell or inadequate response to hematopoietic growth factors, are harder to interpret, although recent genetic studies have suggested explanatory mechanisms (see next paragraph). The reduced number and function of the marrow is secondary to cell destruction, and apoptosis is prevalent among the few remaining elements.^{41,42} Microarray of the scant CD34 cells from marrow failure patients revealed a transcriptome in which genes involved in apoptosis, cell death, and immune regulation were up-regulated⁴³; this transcriptional signature was reproduced in normal CD34 cells exposed to γ -interferon.⁴⁴

One peculiar feature of white blood cells in aplastic anemia is short telomeres.^{45,46} Telomere shortening was initially most easily blamed on stem-cell exhaustion. However, the discovery, first by linkage analysis in large pedigrees, that the X-linked form of dyskeratosis congenita was due to mutations in *DKC1* and subsequently purposeful identification of mutations in *TERC* in some autosomal dominant patients with this constitutional marrow

failure syndrome indicated a genetic basis for telomere deficiency. Central to the repair machinery is an RNA template, encoded by *TERC*, on which telomerase, a reverse transcriptase encoded by *TERT*, elongates the nucleotide repeat structure; other proteins, including the *DKC1* gene product dyskerin, are associated with the telomere repair complex. Systematic surveys of DNA disclosed first *TERC*^{47,48} and later *TERT* mutations⁴⁹ in some patients with apparently acquired aplastic anemia, including older adults. 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; however, their clinical presentation is much later than in typical dyskeratosis congenita, and they lack typical physical anomalies.^{47,49} Chromosomes are also protected by several proteins that bind directly to telomeres, and polymorphisms in their genes (*TERF1*, *TERF2*) are also more or less prevalent in aplastic anemia compared with healthy controls.⁵⁰ A few of our patients also have heterozygous mutations in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene. Almost all children with this form of constitutional aplastic anemia are compound heterozygotes for mutations in *SBDS*, and their white cells have extremely short telomeres⁵¹; however, the *SBDS* gene product has not been directly linked to the telomere repair complex or to telomere binding. A parsimonious inference from all these data is that inherited mutations in genes that repair or protect telomeres are genetic risk factors in acquired aplastic anemia, probably because they confer a quantitatively reduced hematopoietic stem-cell compartment that may also be qualitatively inadequate to sustain immune-mediated damage.⁵²

Telomeres are short in one third to one half of aplastic anemia patients,^{45,46} but mutations have been identified in only less than 10% of cases. The most interesting explanation is involvement of other genes, including genes for other members of the large repair complex, telomere binding proteins, still obscure components of the alternative repair system, and some DNA helicases. Alternatively, telomere shortening may be secondary to stem-cell replication.

Clonal evolution

Clinically, aplastic anemia may coexist or appear to evolve to other hematologic diseases that are characterized by proliferation of distinctive cell clones, as in paroxysmal nocturnal hemoglobinuria (PNH) or myelodysplasia (MDS; Figure 2). The mechanisms linking immune-mediated and premalignant pathophysiologies are not elucidated in marrow failure or in parallel circumstances (chronic hepatitis and hepatocellular carcinoma, ulcerative colitis and colon cancer, and many others). The presence of tiny clones at the time of diagnosis of aplastic anemia, detected using extremely sensitive assays—phenotypic (flow cytometry for PNH) or cytogenetic (fluorescent in situ hybridization for MDS)—also creates the problems of disease classification and patient diagnosis.

PNH. Fifty percent or more of patients at presentation with pancytopenia 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.⁵³ Most clones are small and do not lead to clinical manifestations of hemolysis or thrombosis, but classic PNH can be dominated by marrow failure (the "aplastic anemia/PNH syndrome"), and all PNH patients show evidence of underlying hematopoietic deficiency. The global absence of a large number of cell-surface proteins in PNH has been hypothesized to allow "escape" and survival of a pre-existing mutant clone. Association

of an expanded PNH clone with HLA-DR2²⁹ and with autoantibodies,¹⁸ and as a predictor of responsiveness to immunosuppressive therapies,⁵⁴ suggests that the escape is from immune attack. However, there is little concrete experimental evidence of reproducible differences in either differential immune responsiveness or susceptibility of PNH clones compared with phenotypically normal target-cell populations.^{55,56} In contrast to cells of normal phenotype, the marrow PNH clone retains its proliferative capacity in tissue culture and does not overexpress Fas⁵⁷; comparison by microarray shows that residual cells of normal phenotype in the PNH bone marrow up-regulate the same apoptosis and cell-death genes as do CD34 cells in aplastic marrow, while the PIG-A⁻ clone appears transcriptionally similar to CD34 cells from healthy donors.⁵⁸ Despite a few provocative studies,^{37,55,59} no satisfying mechanism to explain clonal escape has convincing empiric support (see Young⁵³ for review).

MDS. Stereotypical patterns of aneuploidy develop in a minority of patients over time: monosomy 7 or trisomy 8 is most characteristic.⁶⁰ Trisomy 8 is MDS with many immune abnormalities that resemble aplastic anemia. Patients respond to immunosuppressive therapies, and oligoclonal T-cell expansions are usually present.⁶¹ T-cell oligoclonal appear to recognize the aneuploid cells, and specifically WT1 antigen that they express at high levels,⁶² but the target cells are not killed due to up-regulation of antiapoptosis genes, including *c-myc*, *survivin*, and *CDK1*.⁶³ For trisomy 8, abnormal cells targeted by the immune system appear to be selected for their capacity to survive cytotoxic lymphocyte attack.

Monosomy 7 is also a frequent cytogenetic abnormality in aplastic anemia but has a poorer prognosis, with patients usually succumbing to refractory cytopenias or evolving to acute leukemia.⁶⁴ Emergence of monosomy 7 has been linked to exogenous use of G-CSF in aplastic anemia,⁶⁵ as occurs in treated severe congenital neutropenia. Laboratory studies of marrow from aplastic anemia and myelodysplasia patients suggest that monosomy 7 clones expand in an abnormal cytokine milieu: high G-CSF concentrations lead to selection of cells that bear a short isoform of the G-CSF receptor that signals proliferation but not differentiation.⁶⁶

Treatment

Immunosuppression

Antithymocyte globulin (ATG) and cyclosporine (combined or intensive immunosuppression). For aplastic anemia that is severe, as defined by peripheral-blood counts, definitive therapies are immunosuppression or stem-cell transplantation; immunosuppressive therapies are most widely used because of lack of histocompatible sibling donors, patient age, and the immediate cost of transplantation. Even in responding patients, blood counts often

remain below normal but adequate to avoid transfusion and to prevent infection. Most specialists use an ATG-based regimen in combination with cyclosporine, based on the outcomes of relatively large studies performed in the 1990s⁶⁷ (Table 1). The larger experience is with ATG produced in horses, although a rabbit ATG, recently approved for use in the United States, is more potent by weight and in the treatment of graft rejection after solid organ transplantations (a current NIH trial is directly comparing these 2 ATGs as first therapy in severe aplastic anemia). ATG is cytolytic: lymphocyte numbers consistently decline during the first few days of infusion and then return to pretreatment levels in a week or 2. ATGs are produced by immunizing animals against human thymocytes, not lymphocytes, and the mix of antibody specificities plus direct experimentation suggests that ATG may be immunomodulatory as well as lymphocytotoxic, perhaps producing a state of tolerance by preferential depletion of activated T cells. The toxicity of ATG is allergic, related to administration of a heterologous protein, and there is little added infection risk beyond the neutropenia intrinsic to the disease. ATG doses and regimens are empiric and traditional. By administration of a larger dose over fewer days, immune complex formation and consequent serum sickness are minimized, as patients usually do not produce their own antibodies to the foreign protein until a week or 10 days after exposure.

Cyclosporine's selective effect on T-cell function is due to direct inhibition on the expression of nuclear regulatory proteins, resulting in reduced T-cell proliferation and activation. While severe aplastic anemia can respond to cyclosporine alone, it is less effective than either ATG alone or ATG plus cyclosporine.^{73,74} As with ATG, doses and length of treatment have not been formally established. Cyclosporine has many side effects, but most are manageable by dose reduction; permanent kidney damage is unusual with monitoring (to maintain blood levels at nadir of about 200 ng/mL). Maintenance of blood counts may be achieved with very low doses of cyclosporine, such that drug levels in blood are undetectable and toxicity is minimal, even with years of treatment.

Outcomes of combined immunosuppressive therapy. Reported hematologic response rates vary, at least in part due to lack of consensus on parameters (transfusion independence, absolute or relative improvement in blood counts) and defined landmarks. In our experience, improvement of blood counts so that the criteria for severity are no longer met highly correlates with termination of transfusions, freedom from neutropenic infection, and better survival. By this standard, about 60% of patients are responders at 3 or 6 months after initiation of horse ATG.⁷⁰ Comparable figures for hematologic response rates have come from Europe⁶⁹ and Japan.⁷¹ Responders have much better survival prospects than do nonresponders. Long-term prognosis is predicted by the robustness of the early blood count response (defined as either platelets or reticulocytes $> 50 \times 10^9/L$ [50 000/ μL] 3 months after treatment): about

Table 1. Intensive immunosuppression (ATG plus cyclosporine) for severe aplastic anemia

| Study | N | Median age, y | Response, % | Relapse, % | Clonal evolution, % | Survival, % |
|----------------------|-----|---------------|-------------|------------|---------------------|-------------|
| German ⁶⁸ | 84 | 32 | 65 | 19 | 8 | 58 at 11 y |
| EGMBT ⁶⁹ | 100 | 16 | 77 | 12 | 11 | 87 at 5 y |
| NIH ⁷⁰ | 122 | 35 | 61 | 35 | 11 | 55 at 7 y |
| Japan ⁷¹ | 119 | 9 | 68 | 22 | 6 | 88 at 3 y |
| NIH† ⁷² | 104 | 30 | 62 | 37 | 9 | 80 at 4 y |

Only studies of more than 20 enrolled patients are tabulated. Responses to immunosuppressive therapy are usually partial; blood counts may not become normal but transfusions are no longer required and the neutrophil count is adequate to prevent infection. Relapse is usually responsive to further immunosuppressive therapies. Clonal evolution is to dysplastic bone marrow changes and/or cytogenetic abnormalities. For details, see "Immunosuppression."

*With androgens and \pm G-CSF.

†With mycophenolate mofetil.

50% of patients who are treated with horse ATG have a robust response and almost all of them will survive long term. Outcomes of immunosuppressive therapy are related to patient age: 5-year survival of more than 90% of children has been reported in recent German,⁷⁵ Japanese,⁷¹ and Chinese⁷⁶ trials, compared with about 50% survival for adults older than 60 years in the collective European experience.⁷⁷

Relapse, defined conservatively as a requirement for additional immunosuppression and not necessarily recurrent pancytopenia, is not uncommon, occurring in 30% to 40% of responding patients. Relapse defined by renewed need for transfusion was estimated at 12% of European patients at 3 years, but prolonged cyclosporine dependency among all patients was common.⁶⁹ Reinstitution of cyclosporine usually reverses declining blood counts, but when required, a second round of horse⁷⁸ or rabbit⁷⁹ ATG is usually effective. In our experience, relapse does not confer a poor prognosis, but it is obviously inconvenient and may not always be remediable. Molecular analysis of the T-cell response in aplastic anemia, discussed in "Pathophysiology," suggests that the major reason for relapse is incomplete eradication of pathogenic clones by ATG.

More serious than relapse is evolution of aplastic anemia to another clonal hematologic disease, PNH, myelodysplasia, and leukemia. Small PNH clones present at diagnosis usually remain stable over time but may expand sufficiently to produce symptomatic hemolysis. For myelodysplasia and leukemia, the cumulative long-term rate of clonal evolution is about 15%.^{70,80}; evolution is not inevitable in aplastic anemia, and some cytogenetic abnormalities may be transient or, as with trisomy 8, responsive to immunosuppressive treatments. As discussed in "Clonal evolution," emergence of monosomy 7 may be favored in severely neutropenic patients who require chronic G-CSF therapy.⁶⁶

Improving on ATG and cyclosporine. *Growth factors.* Historically, intensification of immunosuppression has increased response rates. However, attempts to improve on ATG plus cyclosporine have been frustratingly disappointing. Megadoses of methylprednisolone only added toxicities. Small pilots of GM-CSF⁸¹ and much larger, randomized studies of G-CSF^{71,82} as routine additions to ATG and cyclosporine have been negative to date; improved neutrophil counts did not translate into a higher rate of recovery or even less infection. A very large ongoing European study of G-CSF should definitively answer efficacy and safety concerns.

Other immunosuppressive drugs. As the addition of cyclosporine clearly improved outcomes compared with the use of ATG alone, other immunosuppressive drugs might be predicted, based on their mode of action, animal studies, and experience in other human diseases and with organ transplantation, to be effective. Mycophenolate mofetil is a tolerizing agent, as it selectively depletes activated cells by inhibition of inosine monophosphate dehydrogenase, a critical enzyme of the purine salvage pathway, therefore blocking activated lymphocyte proliferation. Nonetheless, its addition to ATG and cyclosporine in an NIH trial of 104 patients did not change hematologic response (about 62%), relapse (37%), or evolution rates; at best, there was a modest sparing of cyclosporine usage.⁷² The 4-year survival rate for all treated patients was 80%—almost certainly due to better supportive care rather than any new drug effect.

Sirolimus, which blocks the serine-threonine kinase known as mammalian target of rapamycin is synergistic with cyclosporine in tissue culture and in clinical transplantation. Again, when tested in a randomized protocol in combination with ATG and cyclosporine

in aplastic anemia, results were not superior to these agents only (P.S., unpublished data, January 2006).

Cyclophosphamide. As with ATG, recovery of blood counts can occur after a failed bone marrow transplantation preceded by conditioning with cyclophosphamide. High-dose cyclophosphamide was used intermittently by investigators at Johns Hopkins University (Baltimore, MD) in the 1980s during periods in which ATG apparently was not available to them; in their most recent update, of 38 previously untreated patients, the response rate was 74% and survival estimated at 86%.^{83,84} In contrast, an NIH randomized study was halted early due to the development of fungal infections and a much higher death rate in the cyclophosphamide arm,⁸⁵ and both relapse and cytogenetic evolution were observed.⁸⁶ The major toxicity of high-dose cyclophosphamide, prolonged neutropenia with concomitant susceptibility to infection, is now addressed by the Baltimore investigators by routine antimicrobial prophylaxis and prolonged G-CSF administration. Cyclophosphamide therapy does not eradicate PNH clones, and relapses now have been observed in Baltimore.⁸⁴ In the absence of another randomized trial, comparison of data from a small, single-center pilot with historical and more general results is problematic; it is especially difficult to exclude biased patient selection, both explicit (such as exclusion of those unlikely to respond or with a generally poor prognosis or older patients) and implicit (inability to treat uninsured individuals or foreign citizens).

Management of refractory aplastic anemia. There is no established algorithm for the management of patients who have failed to respond to ATG.⁶⁷ Transplantation from an alternative donor is offered by many centers to children who have failed a single course of immunosuppression and to adults after 2 rounds of ATG therapy (see "Other stem-cell sources"). Response rates to second ATG have ranged from 22% to 64%.⁷⁸ In an Italian study of rabbit ATG as second therapy, 23 (77%) of 30 improved⁸⁷; in the NIH experience, the proportion responding was closer to 30%.⁷⁹ Cyclophosphamide also has been administered in this setting, with a response rate of about 50% reported.⁸³ A third course of immunosuppression may benefit only patients who showed some response to a previous treatment.⁸⁸ Response to retreatment correlates to a better survival compared with refractory patients.⁷⁹ The improved survival of patients who are refractory to immunosuppression, due to better supportive care, complicates the decision to undertake high-risk transplantation (see "Other stem-cell sources"). We have tested alemtuzumab, a humanized monoclonal antibody specific for CD52, an antigen present on all lymphocytes; alemtuzumab induces profound immunosuppression by lymphocytotoxicity and has been effective in lymphoproliferative diseases, graft-versus-host disease (GVHD), and autoimmune disorders. To date, 4 of 8 patients who were refractory to treatment with horse ATG have responded to alemtuzumab, and toxicity has been modest (P.S., unpublished data, January 2006); we are now testing alemtuzumab in a randomized comparison with both horse and rabbit ATG in severe aplastic anemia at presentation.

Treatment of moderate pancytopenia. Clinically, the course of moderate aplastic anemia is variable: some patients progress to severe disease, others remain stable and may not require intervention; regular transfusions may not be required.⁸⁹ 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,⁷³ but in practice are often used sequentially. 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.⁹⁰

When there is residual hematopoietic function, androgens may be effective (although male hormones have failed most rigorous trials in severe aplastic anemia). Some moderate aplastic anemia likely results from telomere gene mutations and stem-cell exhaustion. In vitro, androgens increase telomerase activity in human lymphocytes and CD34 cells, acting through the estradiol receptor,⁹¹ and this activity may provide a mechanism of action for their effects on marrow function.

Hematopoietic stem-cell transplantation

Allogeneic HLA-matched sibling donor transplantation. Hematopoietic and immune system cells are replaced by stem-cell transplantation; conditioning with cyclophosphamide is not myeloablative but is sufficiently immunosuppressive to prevent and to eliminate residual host marrow by a graft-versus-marrow effect.

Allogeneic transplant from a matched sibling donor cures the great majority of patients (Table 2): the most recent cohort reported to the IBMTR showed 77% 5-year survival,¹⁰⁷ and in children, and patients undergoing transplantation who are minimally transfused, survival of 80% to 90% may be routinely achieved. Graft rejection, a historic problem in the application of transplantation to aplastic anemia (most dramatically manifest in rejection of unprepared syngeneic stem cells), is now not frequent in patients who undergo transplantation early and with a modest transfusion burden, likely a benefit of less immunogenic blood products (leukocyte-depleted erythrocytes, for example) from fewer donors (platelets collected by cytopheresis). Conditioning regimens that do not include irradiation now regularly achieve engraftment and avoid many of irradiation's long-term complications, especially late cancers. In a recent series of 81 patients who were prepared by cyclophosphamide plus ATG, sustained engraftment was achieved by 96%, and 3 of the 4 patients who initially rejected the transplant successfully underwent a retransplantation; 88% of the patients survived long term.¹⁰⁴ The combination of cyclophosphamide plus fludarabine, with or without ATG, has achieved high rates of graft acceptance and survival even in heavily transfused patients who received a transplant of mobilized peripheral-blood stem cells, months after proving refractory to immunosuppressive drugs.^{106,108}

Graft-versus-host disease remains a serious problem for older patients, even with routine cyclosporine prophylaxis. In the IBMTR, rates of severe GVHD doubled in adults compared with children (15%-20% for recipients \leq 20 years of age to 40%-45% for $>$ 20 years of age).¹⁰⁷ In Seattle, chronic graft-versus-host disease developed in 41% of patients who had survived more than 2 years after transplantation, tripling the risk of death and often requiring years of immunosuppressive therapy.¹⁰⁹ Even with resolution, chronic GVHD remains a risk factor for late complications such as growth and endocrine system effects, pulmonary disease, cataracts, neurologic dysfunction, and secondary malignancy. Addition of ATG¹⁰⁵ and more recently its substitution by alemtuzumab¹¹⁰ may reduce the frequency and severity of acute GVHD, a predictor of chronic GVHD.

Matched unrelated donor transplant. A matched sibling donor is available in only 20% to 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 (Table 3). Outcomes of 318 alternative donor transplants performed from 1988 to 1998 recently have been summarized for the European registry⁹²: for matched unrelated donors, the rejection rate was 15% and for grades II to IV GVHD, 48%, and 5-year survival was estimated at 39%. From diverse registry data (collected through EBMT, IBMTR, and the National Marrow Donor Program),^{92,107,121} the mortality rate is about twice that observed in matched sibling transplants; even with predominantly younger patients as recipients, age is probably the most powerful influence on survival, but also important are the closeness of the class I HLA match and the length of time from diagnosis. A retrospective analysis from the Japan Marrow Donor Program suggested that patients with the most favorable characteristics and conditioned with a minimal dose of radiation might anticipate survival comparable with matched sibling transplants.¹¹³

Prospective trials have enrolled fewer patients but have better results (perhaps due to superior protocols, but both careful patient selection and publication bias are likely important). In contrast to allogeneic sibling transplants, transplants from unrelated donors still require irradiation to ensure engraftment, due both to source of the donor cells and the transfusion status of

Table 2. Allogeneic sibling transplantation for severe aplastic anemia

| Institution/study | Years of study | N | Age, y (median in y) | Graft rejection/failure, % | Acute GVHD,* % | Chronic GVHD, % | Actuarial survival, % |
|----------------------------|----------------|-----|----------------------|----------------------------|----------------|-----------------|-----------------------|
| IBMTR ⁹² | 1988-1992 | 471 | 20 (1-51) | 16 | 19 | 32 | 66 at 5 y |
| Vienna ⁹³ | 1982-1996 | 20 | 25 (17-37) | 0 | 26 | 53 | 95 at 15 y |
| EBMT ⁹⁴ | 1991-1998 | 71 | 19 (4-46) | 3 | 30 | 35 | 86 at 5 y |
| Seoul ⁹⁵ | 1990-1999 | 22 | 22 (14-43) | 5 | 10 | 33 | 95 at 5 y |
| Seoul ⁹⁶ | 1990-2001 | 64 | 28 (14-43) | 18 | 31 | 19 | 79 at 6 y |
| Hamburg ⁹⁷ | 1990-2001 | 21 | 25 (7-43) | 5 | 5 | 5 | 86 at 5 y |
| Paris ⁹⁸ | 1994-2001 | 33 | 20 (8-42) | 6 | 0 | 42 | 94 at 5 y |
| Sao Paulo ⁹⁹ | 1993-2001 | 81 | 24 (3-53) | 22 | 37 | 39 | 56 at 6 y |
| Taipei ¹⁰⁰ | 1985-2001 | 79 | 22 (4-43) | 8 | 7 | 35 | 74 at 5 y |
| Tunis ¹⁰¹ | 1998-2001 | 31 | 19 (4-39) | 16 | 11 | 3 | 86 at 2 y |
| Seoul ¹⁰² | 1995-2001 | 113 | 28 (16-50) | 15 | 11 | 12 | 89 at 6 y |
| London ¹⁰³ | 1989-2003 | 33 | 17 (4-46) | 24 | 14 | 4 | 81 at 5 y |
| Seattle ^{104,105} | 1988-2004 | 94 | 26 (2-59) | 4 | 24 | 26 | 88 at 6 y |
| Mexico City ¹⁰⁶ | 2000-2005 | 23 | 25 (4-65) | 26 | 17 | 26 | 88 at 4 y |

In contrast to Table 1, response rates are not provided because, in surviving patients who do not experience primary graft rejection or secondary graft failure, full hematologic recovery with donor hematopoiesis is anticipated. Only studies reporting at least 20 patients are tabulated.

GVHD indicates graft-versus-host disease; IBMTR, International Blood and Marrow Transplant Registry; and EBMT, European Group for Bone Marrow Transplant.

*Results are generally for grades II to IV and patients at risk.

Table 3. Alternative donor stem-cell transplantation for severe aplastic anemia

| Study | Year of publication | N | Donor source, N | Conditioning | Age, y, median (range) | Acute GVHD,* % | Chronic GVHD, % | Survival, % |
|---|---------------------|-----|------------------------------|---|------------------------|----------------|-----------------|-------------------|
| Nagoya ¹¹¹ | 2001 | 15 | MUD: 11; MMUD: 4 | Cy/ATG/TBI | 11 (3-19) | 33 | 13 | 100 at 4 y |
| Great Britain ¹¹² | 2001 | 8 | MUD: 7; MMUD: 1 | Cy/CP/TBI | 7 (0-10) | 25 | 0 | 100 at 3 y |
| Japan Marrow Donor Program ¹¹³ | 2002 | 154 | MUD: 79; MMUD: 75 | Cy ± TBI or LFI; Cy/ATG ± TBI or LFI | 17 (1-46) | 29 | 30 | 56 at 5 y |
| Memphis ¹¹⁴ | 2004 | 9 | MUD: 4; MMUD: 5 | High CD34 ⁺ cell dose, TCD, Cy/ATG/TLI or TBI ± thiotepa | 11 (6-16) | 0 | 0 | 89 at 4 y |
| Gyeonggi-do ¹¹⁵ | 2004 | 5 | MUD | Cy/Flu/ATG | 13 (7-18) | 0 | 0 | 80 at 2 y |
| Guangzhou ¹¹⁶ | 2004 | 6 | UCB | Cy/ATG | 26 (22-37) | 0 | 33 | 66 at 2 y |
| Genoa ¹¹⁷ | 2005 | 38 | MUD: 33; MMRD: 5 | Cy/Flu/ATG | 14 (3-37) | 11 | 24 | 73 at 2 y |
| Philadelphia ¹¹⁸ | 2005 | 12 | MUD: 4; MMUD: 8 | Partial TCD, TBI + Cy/ Ara-C or Cy/TT or ATG | 9 (1-20) | 33 | 25 | 75 at 4 y |
| Seoul ¹¹⁹ | 2005 | 13 | MUD: 12; MMUD: 1 | Cy/ATG | 22 (15-34) | 31 | 62 | 75 at 3 y |
| IBMTR ⁹² | 2006 | 318 | MUD: 181; MMRD: 86; MMUD: 51 | Various | 16 (1-55) | 48 for MUD | 29 for MUD | 39 at 5 y for MUD |
| Seattle ¹²⁰ | 2006 | 87 | MUD: 62; MMUD: 25 | Cy/ATG/TBI | 19 (1-53) | 70 for MUD | 52 for MUD | 61 at 5 y for MUD |

Only studies reporting at least 5 patients are tabulated. GVHD, graft-versus-host disease; IBMTR, International Blood and Marrow Transplant Registry; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; Cy, cyclophosphamide; ATG, antithymocyte globulin; TBI, total body irradiation; CP, alemtuzumab; LFI, limited field irradiation; TCD, T-cell depletion; TLI, total lymphoid irradiation; Flu, fludarabine; UCB, umbilical cord blood; MMRD, mismatched related donor; and TT, thiotepa.

*GVHD results are generally for grades II to IV and patients at risk.

the recipient. In a recent multicenter study, 62 patients with severe aplastic anemia who were refractory to immunosuppressive therapy underwent matched unrelated stem-cell transplantation following conditioning with cyclophosphamide, ATG, and total body irradiation; graft failure occurred in 2%, acute grades II to IV GVHD was observed in 70%, chronic GVHD was observed in 52%, and overall survival was 61%. Twenty five patients who lacked an HLA-identical donor received an HLA-nonidentical stem-cell graft: 88% showed sustained engraftment, and overall survival was 44%.¹²⁰ The interval from diagnosis to transplantation in this study did not impact survival.

A European protocol substituted irradiation with fludarabine for unrelated and mismatched family donors: 73% were estimated to survive 2 years; while GVHD rates were relatively low, perhaps due to absence of radiation damage, graft rejection occurred in about one third of the older children and younger adults.¹¹⁷ Children's Hospital of Milwaukee pioneered a rigorous conditioning regimen of cytosine arabinoside, cyclophosphamide, and total body irradiation, which produced long-term survival of about 50% with very little GVHD.¹²² Other single-institution protocols have used a diversity of strategies to improve graft acceptance and reduce GVHD: T-cell depletion, CD34-cell purification, alemtuzumab, chemotherapy and monoclonal antibodies in combination; while almost exclusively enrolling small numbers of children and still preliminary, survival and morbidity may rival results of conventional sibling transplants. In current practice, unrelated transplant is offered for children who have failed a single course of immunosuppression and to adults who are refractory to multiple courses of ATG and alternative therapies such as androgens. Studies with longer follow-up of larger numbers of patients are crucial to establish the optimal conditioning regimen and to define which patients will benefit and especially how early unrelated transplantation should be performed.

Other stem-cell sources. HLA mismatching is better tolerated for umbilical cord transplantations, making them in theory widely applicable. Published data for this procedure in marrow failure is limited, and almost all recipients have been small children, due to

the small numbers of stem cells contained in a single cord-blood sample. In a report from the National Marrow Donor Program, engraftment occurred in less than half of 19 recipients, and almost all the patients died from transplant-related causes or survived due to autologous reconstitution or a second transplantation.¹²³ Surprisingly, 5 of 6 Chinese adult aplastic anemia patients successfully engrafted, and 4 survived.¹¹⁶ Advocates of this approach are using pooled donations to increase stem-cell numbers.

Family members are almost always available to the patient as a stem-cell source, but survival after haploidentical transplantation has been poor. More recently, engraftment was achieved in 3 children using the St Jude protocol, but one ultimately rejected and mixed chimerism in the others required further immunosuppression and donor lymphocyte infusions.¹²⁴

Conclusions and prospects

The treatment of severe aplastic anemia, whether by allogeneic stem-cell transplantation or immunosuppression, has improved dramatically over the last 25 years, and long-term survival of more than 75% of patients can be anticipated with either therapy.¹²⁵ For transplantation, the immediate challenge is the extension of stem-cell replacement to all patients, regardless of age, with a histocompatible sibling, and to others who lack a family donor using alternative stem-cell sources. The ability to achieve engraftment under these difficult circumstances may require conditioning regimens in which complications, particularly second malignancies, may not be apparent for many years. More optimistically, donor selection based on high-resolution histocompatibility typing may improve outcomes. The success of umbilical cord-blood transplantations, with their low risk of GVHD, may be enhanced by larger pools and histocompatibility matching. For immunosuppression, many new drugs and biologics have yet to be tested in aplastic anemia. Again, the costs of intensification need to be balanced against the benefits of higher hematologic response rates and lower rates of relapse and

evolution. Repeated courses of immunosuppression offer the possibility of blood-count restitution to 75% to more than 90% of patients, based on the range of published hematologic recovery rates with initial horse ATG followed by rabbit ATG, alemtuzumab, or cyclophosphamide. If residual stem-cell numbers are limiting, ex vivo expansion of hematopoiesis may be possible, as for example using Hox box proteins. Quantitative and practical measurements of oligoclonal T-cell activity and of hematopoietic stem-cell number and function would allow laboratory testing to guide treatment decisions. Ultimately, definition of genetic risk factors, affecting hematopoietic-cell function and the immune response, will clarify how agents in the

environment initiate and perpetuate the marrow destruction of aplastic anemia.

Acknowledgments

We are thankful to colleagues from the Hematology Branch, Drs A. John Barrett, Cynthia Dunbar, Richard Childs, and Elaine Sloand, and in Europe, Professors Judith Marsh, Gérard Socié, and André Tichelli for their careful reading of the paper and helpful criticisms.

The authors apologize to their colleagues whose papers were not cited in the bibliography due to space constraints.

References

- Young NS. Aplastic anemia. In: Young NS, ed. *The Bone Marrow Failure Syndromes*. Philadelphia, PA: W. B. Saunders; 2000:1-46.
- Kojima S, Nakao S, Tomonaga S, et al. Consensus conference on the treatment of aplastic anemia. *Int J Hematol*. 2000;72:118-123.
- Marsh JCW. Management of acquired aplastic anaemia. *Blood Rev*. 2005;19:143-151.
- Nakao S, Feng X, Sugimori C. Immune pathophysiology of aplastic anemia. *Int J Hematol*. 2005;82:196-200.
- Young NS. Acquired aplastic anemia. In: Young NS, Gerson SL, High K, eds. *Clinical Hematology*. Philadelphia, PA: Elsevier; 2006:136-157.
- Schrezenmeier H, Bacigalupo A. *Aplastic Anemia: Pathophysiology and Treatment*. Cambridge, United Kingdom: Cambridge University Press; 2000.
- Choudhry VP, Gupta S, Gupta M, Kashyap R, Saxena R. Pregnancy associated aplastic anemia: a series of 10 cases with review of literature. *Hematology*. 2002;7:233-238.
- Lu J, Basu A, Melenhorst J, Young NS, Brown KE. Analysis of T-cell repertoire in hepatitis-associated aplastic anemia. *Blood*. 2004;103:4588-4593.
- Morgan GJ, Alvares CL. Benzene and the hematopoietic stem cell. *Chem Biol Interact*. 2005;153-154:217-222.
- Young NS. Drugs and chemicals. In: Young NS, Alter BP, eds. *Aplastic Anemia, Acquired and Inherited*. Philadelphia, PA: W. B. Saunders; 1994:100-132.
- Gerson WT, Fine DG, Spielberg SP, Sensenbrenner LL. Anticonvulsant-induced aplastic anemia: increased susceptibility to toxic drug metabolites in vitro. *Blood*. 1983;61:889-893.
- Sutton JF, Stacey M, Kearsey SE, et al. Increased risk for aplastic anemia and myelodysplastic syndrome in individuals lacking GSTT1 gene. *Pediatric Blood Cancer*. 2004;42:122-126.
- Dufour C, Syahn J, Bacigalupo A, et al. Genetic polymorphisms of CYP3A4, GSTT1, GSTM1, GTP1 and NQO1 and the risk of acquired idiopathic aplastic anemia in Caucasian patients. *Haematologica*. 2005;90:1027-1031.
- Bacigalupo A. Aetiology of severe aplastic anaemia and outcome after allogeneic bone marrow transplantation or immunosuppression. *Eur J Haematol*. 1996;57(suppl 60):16-19.
- Kaufman DW, Kelly JP, Levy M, Shapiro S. *The Drug Etiology of Agranulocytosis and Aplastic Anemia*. New York, NY: Oxford; 1991.
- Issaragrisil S, Kaufman D, Anderson T, et al. The epidemiology of aplastic anemia in Thailand. *Blood*. 2006;107:1299-1307.
- Hirano N, Butler MO, von Bergwelt-Baildon MS, et al. Autoantibodies frequently detected in patients with aplastic anemia. *Blood*. 2003;102:4567-4575.
- Feng X, Chuhjo T, Sugimori C, et al. Diazepam-binding inhibitor-related protein 1: a candidate autoantigen in acquired aplastic anemia patients harboring a minor population of paroxysmal nocturnal hemoglobinuria-type cells. *Blood*. 2004;104:2425-2431.
- Hinterberger W, Rowlings PA, Hinterberger-Fischer M, et al. Results of transplanting bone marrow from genetically identical twins into patients with aplastic anemia. *Ann Intern Med*. 1997;126:116-122.
- Young NS. Hematopoietic cell destruction by immune mechanisms in acquired aplastic anemia. *Semin Hematol*. 2000;37:3-14.
- Sloand EM, Kim S, Maciejewski JP, et al. Intracellular interferon- γ in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia. *Blood*. 2002;100:1185-1191.
- Risitano AM, Maciejewski JP, Green S, et al. In vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenic T-cell clones by TCR beta-CDR3 sequencing. *Lancet*. 2004;364:355-364.
- Maciejewski JP, Selleri C, Anderson S, Young NS. Fas antigen expression on CD34+ human marrow cells is induced by interferon-gamma and tumor necrosis factor-alpha and potentiates cytokine-mediated hematopoietic suppression in vitro. *Blood*. 1995;85:3183-3190.
- Selleri C, Maciejewski JP, Sato T, Young NS. Interferon- γ constitutively expressed in the stromal microenvironment of human marrow cultures mediates potent hematopoietic inhibition. *Blood*. 1996;87:4149-4157.
- Bloom ML, Wolk A, Simon-Stoos K, et al. A mouse model of human immune mediated bone marrow failure. *Exp Hematol*. 2004;32:1163-1172.
- Chen J, Lipovsky K, Ellison FM, Calado RT, Young NS. Bystander destruction of hematopoietic progenitor and stem cells in a mouse model of infusion-induced bone marrow failure. *Blood*. 2004;104:1671-1678.
- Chen J, Young NS. A murine model of bone marrow failure mediated by disparity in minor histocompatibility antigens [abstract]. *Blood*. 2004;106:12a. Abstract 132.
- Nakao S, Takamatsu H, Chuhjo T, et al. Identification of a specific HLA class II haplotype strongly associated with susceptibility to cyclosporine-dependent aplastic anemia. *Blood*. 1994;84:4257-4261.
- Maciejewski JP, Follmann D, Rivera CE, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and PNH/aplastic anemia syndrome. *Blood*. 2001;98:3513-3519.
- Peng J, Liu C, Zhu K, et al. The TNF2 allele is a risk factor to severe aplastic anemia independent of HLA-DR. *Hum Immunol*. 2003;64:896-901.
- Demeter J, Messer G, Schrezenmeier H. Clinical relevance of the TNF-alpha promoter/enhancer polymorphism in patients with aplastic anemia. *Ann Hematol*. 2002;81:566-569.
- Dufour C, Capasso M, Svahn J, et al. Homozygosity for (12) CA repeats in the first intron of the human IFN-gamma gene is significantly associated with the risk of aplastic anaemia in Caucasian population. *Br J Haematol*. 2004;126:682-685.
- Gidvani V, Ramkissoon S, Wong E, et al. Tumor necrosis factor-alpha and interleukin-6 promoter gene polymorphisms in acquired bone marrow failure syndromes [abstract]. *Proceedings of the 46th ASH Annual Meeting*. 2004;104:12b. Abstract 3707.
- Solomou EE, Keyvanfar K, Young NS. T-bet, a Th1 transcription factor, is up-regulated in T cells from patients with aplastic anemia. *Blood*. 2006;107:3983-3991.
- Xu JL, Nagasaka T, Nakashima N. Involvement of cytotoxic granules in the apoptosis of aplastic anaemia. *Br J Haematol*. 2003;120:850-852.
- Zeng W, Kajigaya S, Chen G, et al. Transcript profile of CD4+ and CD8+ cells from the bone marrow of acquired aplastic anemia patients. *Exp Hematol*. 2004;32:806-814.
- Poggi A, Negrini S, Zocchi MR, et al. Patients with paroxysmal nocturnal hemoglobinuria have a high frequency of peripheral-blood T cells expressing activating isoforms of inhibiting superfamily receptors. *Blood*. 2005;106:2399-2408.
- Howe EC, Wlodarski M, Ball EJ, Rybicki L, Maciejewski JP. Killer immunoglobulin-like receptor genotype in immune-mediated bone marrow failure syndromes. *Exp Hematol*. 2005;33:1357-1362.
- Maciejewski JP, Selleri C, Tadatsugu S, Anderson S, Young NS. A severe and consistent deficit in marrow and circulating primitive hematopoietic cells (long-term culture-initiating cells) in acquired aplastic anemia. *Blood*. 1996;88:1983-1991.
- Schrezenmeier H, Jenal M, Herrmann F, Heimpel H, Raghavachar A. Quantitative analysis of cobblestone area-forming cells in bone marrow of patients with aplastic anemia by limiting dilution assay. *Blood*. 1996;88:4474-4480.
- Philpott NJ, Scopes J, Marsh JCW, Gordon-Smith EC, Gibson FM. Increased apoptosis in aplastic anemia bone marrow progenitor cells: possible pathophysiological significance. *Exp Hematol*. 1995;23:1642-1648.
- Kakagianni T, Giannakoulas NC, Thanopoulou E, et al. A probable role for trail-induced apoptosis in the pathogenesis of marrow failure: implications from an in vitro model and from marrow of aplastic anemia patients. *Leuk Res*. 2006;30:713-721.
- Zeng W, Chen G, Kajigaya S, et al. Gene expression profiling in CD34 cells to identify differences between aplastic anemia patients and healthy volunteers. *Blood*. 2004;103:325-332.
- Zeng W, Miyazato A, Chen G, Kajigaya S, Young NS. Interferon- γ -induced gene expression in

- CD34 cells: identification of pathologic cytokine-specific signature profiles. *Blood*. 2006;107:167-175.
45. Ball SE, Gibson FM, Rizzo S, et al. Progressive telomere shortening in aplastic anemia. *Blood*. 1998;91:3582-3592.
 46. Brummendorf TH, Maciejewski JP, Young NS, Lansdorp PL. Telomere length in leukocyte subpopulations of patients with aplastic anemia. *Blood*. 2001;97:895-900.
 47. Fogarty PF, Yamaguchi H, Wiestner A, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. *Lancet*. 2003;362:1628-1630.
 48. Yamaguchi H, Baerlocher GM, Lansdorp PL, et al. Mutations of the human telomerase RNA gene (TERC) in aplastic anemia and myelodysplastic syndrome. *Blood*. 2003;102:916-918.
 49. Yamaguchi H, Calado RT, Ly H, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. *N Engl J Med*. 2005;352:1413-1424.
 50. Savage SA, Calado RT, Lansdorp PM, Chanock SJ, Young NS. Genetic variation in telomeric repeat binding factor 1 but not telomeric repeat binding factor 2 are associated with aplastic anemia. *Exp Hematol*. 2006;34:664-671.
 51. Thornley I, Dror Y, Sung L, Wynn RF, Freedman MH. Abnormal telomere shortening in leukocytes of children with Shwachman-Diamond syndrome. *Br J Haematol*. 2002;117:189-192.
 52. Calado RT, Bruno T, Wilkerson KL, Young NS. Evidence for T-cell oligoclonal expansion in aplastic anemia associated with telomerase complex mutations: pathophysiological and clinical implications [abstract]. *Blood*. 2005;106:307a. Abstract 1052.
 53. Young NS. Paroxysmal nocturnal hemoglobinuria: current issues in pathophysiology and treatment. *Curr Hematol Rep*. 2005;4:103-109.
 54. Sugimori C, Chuhjo T, Feng X, et al. Minor population of CD55-CD59 blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. *Blood*. 2006;107:1308-1314.
 55. Takami A, Zeng W, Wang H, Matsuda T, Nakao S. Cytotoxicity against lymphoblastoid cells mediated by a T-cell clone from an aplastic anaemia patient: role of CD59 on target cells. *Br J Haematol*. 1999;107:791-796.
 56. Karadimitris A, Notaro R, Koehne G, Roberts IAG, Luzzatto L. PNH cells are as sensitive to T-cell-mediated lysis as their normal counterparts: implications for the pathogenesis of paroxysmal nocturnal haemoglobinuria. *Brit J Haematol*. 2000;111:1158-1163.
 57. Chen G, Kirby M, Zeng W, Young NS, Maciejewski JP. Superior growth of glycoposphatidy inositol-anchored protein-deficient progenitor cells in vitro is due to the higher apoptotic rate of progenitors with normal phenotype in vivo. *Exp Hematol*. 2002;30:774-782.
 58. Chen G, Zeng W, Maciejewski JP, et al. Differential gene expression profile in hematopoietic progenitors from paroxysmal nocturnal hemoglobinuria patients reveals an apoptosis/immune response in 'normal' phenotype cells. *Leukemia*. 2005;19:862-868.
 59. Hanaoka N, Kawaguchi T, Horikawa K, et al. Immunoselection by natural killer cells of PIGA mutant cells missing stress-inducible ULBP. *Blood*. 2006;107:1184-1191.
 60. Maciejewski JP, Selleri C. Evolution of clonal cytogenetic abnormalities in aplastic anemia. *Leuk Lymphoma*. 2004;45:433-440.
 61. Sloand EM, Fuhrer M, Risitano A, et al. Preferential suppression of trisomy 8 versus normal hematopoietic cell growth by autologous lymphocytes in patients with trisomy 8 myelodysplastic syndrome. *Blood*. 2005;106:841-851.
 62. Sloand EM, Rezvani K, Mainwaring L, et al. Myelodysplasia with trisomy 8 is associated with a cytotoxic CD8 T cell immune response to Wilms tumor-1 protein (WT1) [abstract]. *Blood*. 2004;104:138a. Abstract 474.
 63. Sloand EM, Mainwaring L, Tarnowka MK, Barrett AJ, Young NS. CD34 cells from patients with trisomy 8 express early apoptotic markers but do not undergo programmed cell death because of upregulation of anti-apoptotic proteins [abstract]. *Blood*. 2003;102:145a. Abstract 497.
 64. Maciejewski JP, Risitano AM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. *Blood*. 2002;99:3129-3135.
 65. Kojima S, Ohara A, Tsuchida M, et al. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. *Blood*. 2002;100:786-790.
 66. Sloand EM, Yong A, Solomou EE, et al. Granulocyte colony-stimulating factor preferentially stimulates proliferation of monosomy 7 cells bearing the isoform IV receptor. *Proc Natl Acad Sci U S A*. In press.
 67. Frickhofen N, Rosenfeld SJ. Immunosuppressive treatment of aplastic anemia with antithymocyte globulin and cyclosporine. *Semin Hematol*. 2000;37:56-68.
 68. Frickhofen N, Heimpel H, Kaltwasser JP, Schrezenmaier H. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. *Blood*. 2003;101:1236-1242.
 69. Bacigalupo A, Bruno B, Saracco P, et al. Antilymphocyte globulin, cyclosporine, prednisolone, and granulocyte colony-stimulating factor for severe aplastic anemia: an update of the GITMO/EBMT study on 100 patients: European Group for Blood and Marrow Transplantation (EBMT) Working Party on Severe Aplastic Anemia and the Gruppo Italiano Trapianti di Midollo Osseo (GITMO). *Blood*. 2000;95:1931-1934.
 70. Rosenfeld S, Follman D, Nunez O, Young NS. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. *JAMA*. 2003;289:1130-1135.
 71. Kojima S, Hibi S, Kosaka Y, et al. Immunosuppressive therapy using antithymocyte globulin, cyclosporine, and danazol with or without human granulocyte colony-stimulating factor in children with acquired aplastic anemia. *Blood*. 2000;96:2049-2054.
 72. Scheinberg P, Nunez O, Wu C, Young NS. Treatment of severe aplastic anaemia with combined immunosuppression: antithymocyte globulin, ciclosporin, and mycophenolate mofetil. *Br J Haematol*. 2006;133:606-611.
 73. Marsh J, Schrezenmaier H, Marin P, et al. Prospective randomized multicenter study comparing cyclosporin alone versus the combination of antithymocyte globulin and cyclosporin for treatment of patients with nonsevere aplastic anemia: a report from the European Blood and Marrow Transplant (EBMT) Severe Aplastic Anemia Working Party. *Blood*. 1999;93:2191-2195.
 74. Raghavachar A, Kolbe K, Höffken K, et al. A randomized trial of standard immunosuppression versus cyclosporine and filgrastim in severe aplastic anemia [abstract]. *Blood*. 1997;90:439a. Abstract 1951.
 75. Fuhrer M, Rampf U, Baumann I, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. *Blood*. 2005;106:2102-2104.
 76. Fang JP, Xu HG, Huang SL, Chen C, Huang K. Immunosuppressive treatment of aplastic anemia in Chinese children with antithymocyte globulin and cyclosporine. *Pediatr Hematol Oncol*. 2006;23:45-50.
 77. Tichelli A, Socié G, Henry-Amar M, et al. Effectiveness of immunosuppressive therapy in older patients with aplastic anemia: The European Group for Blood and Marrow Transplantation Severe Aplastic Anaemia Working Party. *Ann Intern Med*. 1999;130:193-201.
 78. Tichelli A, Passweg J, Nissen C, et al. Repeated treatment with horse antilymphocyte globulin for severe aplastic anaemia. *Br J Haematol*. 1998;100:393-400.
 79. Scheinberg P, Nunez O, Young NS. Retreatment with rabbit antithymocyte globulin and cyclosporine for patients with relapsed or refractory severe aplastic anemia. *Br J Haematol*. 2006;133:622-627.
 80. Socié G, Rosenfeld S, Frickhofen N, Gluckman E, Tichelli A. Late clonal diseases of treated aplastic anemia. *Semin Hematol*. 2000;37:91-101.
 81. Jeng MR, Naidu PE, Rieman MD, et al. Granulocyte-macrophage colony stimulating factor and immunosuppression in the treatment of pediatric acquired severe aplastic anemia. *Pediatr Blood Cancer*. 2005;45:170-175.
 82. Gluckman E, Rokicka-Milewska R, Hann I, et al. Results and follow-up of a phase III randomized study of recombinant human-granulocyte stimulating factor as support for immunosuppressive therapy in patients with severe aplastic anaemia. *Br J Haematol*. 2002;119:1075-1082.
 83. Brodsky RA, Sensenbrenner LL, Smith BD, et al. Durable treatment-free remission after high dose cyclophosphamide therapy for previously severe aplastic anemia. *Ann Intern Med*. 2001;135:477-483.
 84. Brodsky RA, Chen A, Dorr DM, Brodsky I, Jones RJ. High dose cyclophosphamide (CY) for severe aplastic anemia (SAA): safety and long term follow-up [abstract]. *Blood*. 2005;106:41a-42a. Abstract 129.
 85. Tisdale JF, Dunn DE, Geller NL, et al. High-dose cyclophosphamide in severe aplastic anemia: a randomized trial. *Lancet*. 2000;356:1554-1559.
 86. Tisdale JF, Maciejewski JP, Nunez O, Rosenfeld SJ, Young NS. Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial. *Blood*. 2002;100:4668-4670.
 87. Di Bona E, Rodeghiero F, Bruno B, et al. Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a first course of intensive immunosuppressive therapy. Gruppo Italiano Trapianti di Midollo Osseo (GITMO). *Br J Haematol*. 1999;107:330-334. (Erratum in: *Br J Haematol*. 2000;108:461.)
 88. Gupta V, Gordon-Smith EC, Cook G, et al. A third course of anti-thymocyte globulin in aplastic anaemia is only beneficial in previous responders. *Br J Haematol*. 2005;129:110-117.
 89. Howard SC, Naidu PE, Hu XJ, et al. Natural history of moderate aplastic anemia in children. *Pediatr Blood Cancer*. 2004;43:545-551.
 90. Maciejewski JP, Sloand EM, Nunez O, Boss C, Young NS. Recombinant humanized anti-IL-2 receptor antibody (Daclizumab) produces responses in patients with moderate aplastic anemia. *Blood*. 2003;102:3584-3586.
 91. Calado RT, Yewdell WT, Wilkerson KL, Kajigaya S, Young NS. Sex hormones up-regulate telomerase activity of normal human hematopoietic cells and restore telomerase activity in carriers of telomerase complex mutations [abstract]. *Blood*. 2005;106:641a. Abstract 2276.
 92. Passweg JR, Perez WS, Eapen M, et al. Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia. *Bone Marrow Transplant*. 2006;37:641-649.

93. Reiter E, Keil F, Brugger S, et al. Excellent long-term survival after allogeneic marrow transplantation in patients with severe aplastic anemia. *Bone Marrow Transplant.* 1997;19:1191-1196.
94. Locatelli F, Bruno B, Zecca M, et al. Cyclosporin A and short-term methotrexate versus cyclosporin A as graft versus host disease prophylaxis in patients with severe aplastic anemia given allogeneic bone marrow transplantation from an HLA-identical sibling: results of a GITMO/EBMT randomized trial. *Blood.* 2000;96:1690-1697.
95. Kim I, Yoon SS, Park S, Kim BK, Kim NK. The treatment of severe aplastic anemia: outcomes of bone marrow transplantation and immunosuppressive therapy in a single institution of Korea. *J Korean Med Sci.* 2003;18:365-371.
96. Ahn MJ, Choi JH, Lee YY, et al. Outcome of adult severe or very severe aplastic anemia treated with immunosuppressive therapy compared with bone marrow transplantation: multicenter trial. *Intl J Hematol.* 2003;78:133-138.
97. Kröger N, Zabelina T, Renges H, et al. Long-term follow-up of allogeneic stem cell transplantation in patients with severe aplastic anemia after conditioning with cyclophosphamide plus antithymocyte globulin. *Ann Hematol.* 2002;81:627-631.
98. Ades L, Mary JY, Robin M, et al. Long-term outcome after bone marrow transplantation for severe aplastic anemia. *Blood.* 2004;103:2490-2497.
99. Dulley FL, Vigorito AC, Aranha FJP, et al. Addition of low-dose busulfan to cyclophosphamide in aplastic anemia patients prior to allogeneic bone marrow transplantation to reduce rejection. *Bone Marrow Transplant.* 2004;33:9-13.
100. Bai LY, Chiou TJ, Liu JH, et al. Hematopoietic stem cell transplantation for severe aplastic anemia: experience of an institute in Taiwan. *Ann Hematol.* 2004;83:38-43.
101. Abdelkefi A, Ben Othman T, Ladeb S, et al. Bone marrow transplantation for patients with acquired severe aplastic anemia using cyclophosphamide and antithymocyte globulin: the experience from a single center. *Hematol J.* 2003;4:208-213.
102. Kim HJ, Park CY, Park YH, et al. Successful allogeneic hematopoietic stem cell transplantation using triple agent immunosuppression in severe aplastic anemia patients. *Bone Marrow Transplant.* 2003;31:79-86.
103. Gupta V, Ball SE, Yi Q, et al. Favorable effect on acute and chronic graft-versus-host disease with cyclophosphamide and in vivo anti-CD52 monoclonal antibodies for marrow transplantation from HLA-identical sibling donors for acquired aplastic anemia. *Biol Blood Bone Marrow Transplant.* 2004;10:867-876.
104. Kahl C, Leisenring W, Deeg HJ, et al. Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up. *Br J Haematol.* 2005;130:747-751.
105. Storb R, Blume KG, O'Donnell MR, et al. Cyclophosphamide and antithymocyte globulin to condition patients with aplastic anemia for allogeneic marrow transplantations: the experience in four centers. *Biol Blood Marrow Transplant.* 2001;7:39-44.
106. Gomez-Almaguer D, Vela-Ojeda J, Jaime-Perez JC, et al. Allografting in patients with severe, refractory aplastic anemia using peripheral blood stem cells and a fludarabine-based conditioning regimen: the Mexican experience. *Am J Hematol.* 2006;81:157-161.
107. Horowitz MM. Current status of allogeneic bone marrow transplantation in acquired aplastic anemia. *Semin Hematol.* 2000;37:30-42.
108. Srinivasan R, Takahashi Y, McCoy JP, et al. Overcoming graft rejection in heavily transfused and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation. *Br J Haematol.* 2006;133:305-314.
109. Deeg HJ, Leisenring W, Storb R, et al. Long-term outcome after marrow transplantation for severe aplastic anemia. *Blood.* 1998;91:3637-3645.
110. Gupta V, Ball SE, Sage D, et al. Marrow transplants from matched unrelated donors for aplastic anaemia using alemtuzumab, fludarabine and cyclophosphamide based conditioning. *Bone Marrow Transplant.* 2005;35:467-471.
111. Kojima S, Inaba J, Yoshimi A, et al. Unrelated donor marrow transplantation in children with severe aplastic anaemia using cyclophosphamide, anti-thymocyte globulin and total body irradiation. *Br J Haematol.* 2001;114:706-711.
112. Vassiliou GS, Webb DKH, Pamphilon D, Knapper S, Veys PA. Improved outcome of alternative donor bone marrow transplantation in children with severe aplastic anaemia using a conditioning regimen containing low-dose total body irradiation, cyclophosphamide and Campath. *Br J Haematol.* 2001;114:701-705.
113. Kojima S, Matsuyama T, Kato S, et al. Outcome of 154 patients with severe aplastic anemia who received transplants from unrelated donors: the Japan Marrow Donor Program. *Blood.* 2002;100:799-805.
114. Benesch M, Urban C, Sykora KW, et al. Transplantation of highly purified CD34+ progenitor cells from alternative donors in children with refractory severe aplastic anaemia. *Br J Haematol.* 2004;125:58-63.
115. Kang HJ, Shin HY, Choi HS, Ahn HS. Fludarabine, cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. *Bone Marrow Transplant.* 2004;34:939-943.
116. Mao P, Wang S, Wang S, et al. Umbilical cord blood transplant for adult patients with severe aplastic anemia using anti-lymphocyte globulin and cyclophosphamide as conditioning therapy. *Bone Marrow Transplant.* 2004;33:33-38.
117. Bacigalupo A, Locatelli F, Lanino E, et al. Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party. *Bone Marrow Transplant.* 2005;36:947-950.
118. Bunin N, Aplenc R, Iannone R, et al. Unrelated donor bone marrow transplantation for children with severe aplastic anemia: minimal GVHD and durable engraftment with partial T-cell depletion. *Bone Marrow Transplant.* 2006;37:143-149.
119. Lee J-H, Choi S-J, Lee J-H, et al. Non-total body irradiation containing preparative regimen in alternative donor bone marrow transplantation for severe aplastic anemia. *Bone Marrow Transplant.* 2005;35:755-761.
120. Deeg HJ, O'Donnell M, Tolar J, et al. Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy. *Blood.* 2006;108:1485-1491.
121. Deeg HJ, Seidel K, Casper J, et al. Marrow transplantation from unrelated donors for patients with severe aplastic anemia who have failed immunosuppressive therapy. *Biol Blood Marrow Transplant.* 1999;5:243-252.
122. Margolis DA, Casper JT. Alternative-donor hematopoietic stem-cell transplantation for severe aplastic anemia. *Semin Hematol.* 2000;37:43-55.
123. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1997;339:1565-1576.
124. Woodard P, Cunningham JM, Benaim E, et al. Effective donor lymphohematopoietic reconstitution after haploidentical CD34+ selected hematopoietic stem cell transplantation in children with refractory severe aplastic anemia. *Bone Marrow Transplant.* 2004;33:411-418.
125. Bacigalupo A, Brand R, Oneto R, et al. Treatment of acquired severe aplastic anemia: bone marrow transplantation compared with immunosuppressive therapy: The European Group for Blood and Marrow Transplantation experience. *Semin Hematol.* 2000;37:69-80.
126. Ehrlich P. Ueber einem Fall von Anämie mit Bemerkungen über regenerative Veränderungen des Knochenmarks. *Charité-Annalen.* 1888;13:300-309.



blood[®]

2006 108: 2509-2519
doi:10.1182/blood-2006-03-010777 originally published
online June 15, 2006

Current concepts in the pathophysiology and treatment of aplastic anemia

Neal S. Young, Rodrigo T. Calado and Phillip Scheinberg

Updated information and services can be found at:
<http://www.bloodjournal.org/content/108/8/2509.full.html>

Articles on similar topics can be found in the following Blood collections

[Clinical Trials and Observations](#) (4364 articles)
[Immunobiology](#) (5399 articles)
[Red Cells](#) (1159 articles)
[Reviews in Translational Hematology](#) (58 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
<http://www.bloodjournal.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:
<http://www.bloodjournal.org/site/subscriptions/index.xhtml>