Acquired Aplastic Anemia
Neal S. Young, MD

In aplastic anemia, hematopoiesis fails: Blood cell counts are extremely low, and the bone marrow appears empty. The pathophysiology of aplastic anemia is now believed to be immune-mediated, with active destruction of blood-forming cells by lymphocytes. The aberrant immune response may be triggered by environmental exposures, such as to chemicals and drugs or viral infections and, perhaps, endogenous antigens generated by genetically altered bone marrow cells. In patients with post-hepatitis aplastic anemia, antibodies to the known hepatitis viruses are absent; the unknown infectious agent may be more common in developing countries, where aplastic anemia occurs more frequently than it does in the West.

The syndrome paroxysmal nocturnal hemoglobinuria (PNH) is intimately related to aplastic anemia because many patients with bone marrow failure have an increased population of abnormal cells. In PNH, an entire class of proteins is not displayed on the cell surface because of an acquired X-chromosome gene mutation. The PNH cells may have a selective advantage in resisting immune attack. In contrast, the disease myelodysplasia can be confused with aplasia and can also evolve from aplastic anemia. The occurrence of cytogenetic abnormalities in patients years after presentation implies that genomic instability is a feature of this immune-mediated disease.

Aplastic anemia can be effectively treated by stem-cell transplantation or immunosuppressive therapy. Transplantation is curative but is best used for younger patients who have histocompatible sibling donors. Antithymocyte globulin and cyclosporine restore hematopoiesis in approximately two thirds of patients. However, recovery of blood cell count is often incomplete, recurrent pancytopenia requires retreatment, and some patients develop late complications (especially myelodysplasia).

In aplastic anemia’s long history, from its early description by Ehrlich (1) at the end of the 19th century, and the simplicity of its pathology, an empty bone marrow, have made it the paradigm of hematopoietic failure syndromes. Aplastic anemia is now increasingly recognized as being closely related to other hematologic diseases (Figure 1). Erythrocytes, granulocytes, and platelets, which are normally produced in the bone marrow, decrease to dangerously low levels. Blood cell counts determine presentation and prognosis. Anemia leads to fatigue, dyspnea, and cardiac symptoms; thrombocytopenia to bruising and mucosal bleeding; and neutropenia to sharply increased susceptibility to infection. When patients are treated with transfusions and antibiotics alone, survival rates are poor and related to the severity of the pancytopenia, as defined by the presence of two of three criteria: a neutrophil count less than \(0.5 \times 10^9\) cells/L, a platelet count less than \(20 \times 10^9\) cells/L, and a reticulocyte count less than 1%. When the neutrophil count is less than \(0.2 \times 10^9\) cells/L, the disease is characterized as very severe. In the early 20th century, patients often died quickly of heart failure, profound hemorrhage, or overwhelming infection. In the modern era of erythrocyte and platelet transfusions, the most common causes of death are recurrent bacterial sepsis or fungal invasion of critical organs secondary to refractory granulocytopenia.

Historically, aplastic anemia has been strongly associated with exposure to chemicals and drugs in the environment, giving the disease a social impact disproportionate to its incidence (2). The recognition of bone marrow failure in workers exposed to benzene led to heroic industrial hygiene crusades by Alice Hamilton and Harrison Martland in the United States in the 1920s and 1930s. In the late 1940s and early 1950s, an epidemic of aplastic anemia appeared to follow the introduction of chloramphenicol, and the disease has been linked to many classes of pharmaceuticals widely used in medical practice (Table). Because aplastic anemia has become such a feared disorder as a result of its association with common drug use, even a few cases can have a profound effect on new drug development by the pharmaceutical industry. Also, this believed association with numerous, diverse possible causes, from chemicals and drugs to hepatitis, infectious mononucleosis, pregnancy, and collagen vascular processes (for example, eosinophilic fasciitis), has led to the belief that there also are numerous and different mechanisms of disease.

However, we now have a plausible, unified model of the pathophysiology of aplastic anemia, drawn from both compelling clinical observations of therapeutic efficacy and systematic laboratory experimentation. The early, successful use of bone marrow transplantation to cure aplastic anemia implicated a stem-cell deficiency.
Later, responses to immunosuppressive therapies pointed to an immune mechanism of hematopoietic failure. As aplastic anemia is progressively demystified, questions of some biological interest emerge. These are relevant to bone marrow failure as well as to our conceptions of autoimmune diseases of other organ systems and to the relationship of immune mechanisms to malignant transformation.

**Immune Pathophysiology of Aplastic Anemia**

Most cases of acquired aplastic anemia can be pathophysiologically characterized as T-cell–mediated, organ-specific destruction of bone marrow hematopoietic cells (4). In an individual patient, the aberrant immune response can sometimes be linked to a viral infection or to drug or chemical exposure (Figure 2). There is much less evidence for other mechanisms, such as direct toxicity for stem cells or a deficiency of stromal-cell or hematopoietic growth factor function. Furthermore, the variability in clinical course and response to treatment can be explained by the quantitative degree of stem-cell destruction and qualitative variations in immune response.

### Hematopoietic Failure

That failure of blood cell production was responsible for the empty bone marrow was a prescient conclusion of the earliest observers of the “yellow fat” of the bony spaces and the absence of the morphologically diverse precursors of mature blood elements—still so striking on examination of bone marrow aspirate smears or core biopsy specimens (5). Magnetic resonance imaging of the vertebrae shows uniform replacement of marrow with fat. Immature hematopoietic cells can also be quantitated by fluorescent-activated flow cytometry, which can detect the CD34 cell antigen, an adhesion protein present on less than 1% of normal bone marrow. CD34 cells are almost absent in

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**Figure 1.** Venn diagram showing possible relationships among bone marrow failure syndromes.

These syndromes include clonal diseases (paroxysmal nocturnal hemoglobinuria, myelodysplasia, and large granular lymphocytosis); and single hematopoietic lineage deficiency diseases (agranulocytosis, pure red-cell aplasia, and amegakaryocytic thrombocytopenia); note especially the areas of overlap between aplastic anemia and paroxysmal nocturnal hemoglobinuria and myelodysplasia.
Acquired Aplastic Anemia

Table. Drugs Associated with Aplastic Anemia in the International Aplastic Anemia Agranulocytosis Study*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Stratified Risk Estimate (95% CI)</th>
<th>Multivariate Relative Risk Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsteroidal analgesics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butazones</td>
<td>3.7 (1.9–7.2)</td>
<td>5.1 (2.1–12)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>7.1 (3.4–15)</td>
<td>8.2 (3.3–20)</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>9.8 (3.3–29)</td>
<td>7.4 (2.1–26)</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>4.6 (2.0–11)</td>
<td>4.2 (1.6–11)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonamides†</td>
<td>2.8 (1.1–7.3)</td>
<td>2.2 (0.6–7.4)</td>
</tr>
<tr>
<td>Antithyroid drugs</td>
<td>16 (4.8–54)</td>
<td>11 (2.0–56)</td>
</tr>
<tr>
<td>Cardiovascular drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>3.3 (1.6–7.0)</td>
<td>3.1 (1.2–8.0)</td>
</tr>
<tr>
<td>Psychotropic drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenothiazines</td>
<td>3.0 (1.1–8.2)</td>
<td>1.6 (0.4–7.4)</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>5.0 (2.8–8.9)</td>
<td>3.5 (1.6–7.7)</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>7.3 (3.0–17)</td>
<td>5.9 (1.8–19)</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>29 (9.7–89)</td>
<td>–</td>
</tr>
</tbody>
</table>

* Relative risk quantitates the relative increase in the incidence of aplastic anemia in users of specific drugs compared with nonusers. The absolute risk, however, for developing aplastic anemia as a result of drug use is very small. Aplastic anemia is rare and is a very infrequent complication of drug use relative to the large number of people using drugs. Adapted with permission from Kaufman et al. (3).
† Does not include trimethoprim–sulfonamide combination.
‡ No multivariate relative risk estimate because of insufficient number of exposed patients and controls.

Aplastic anemia. Progenitor cells capable of forming erythroid, myeloid, and megakaryocytic colonies in tissue culture are greatly reduced, and assays of very primitive, quiescent, hematopoietic cells that are closely related if not identical to stem cells show a similar consistent and severe deficit. By extrapolation from such functional studies of aplastic bone marrow, it is likely that patients present with pancytopenia when stem-cell and progenitor-cell populations have decreased to approximately 1% or less of normal. Such a profound deficiency has important qualitative consequences, as reflected in the shortened telomere length of granulocytes of patients with aplastic anemia (6).

Immune Destruction

The efficiency of immune system destruction of blood-forming cells is obvious in “runt disease” in animals and in transfusion-associated graft-versus-host disease (GVHD) in humans (7). In these syndromes, small numbers of alloreactive T cells produce fatal aplastic anemia, and in the mouse model, we know that stem-cell destruction is rapid and almost complete. Much laboratory data support the hypothesis that, in most patients with acquired aplastic anemia, lymphocytes are responsible for the destruction of the hematopoietic cell compartment (4).

Early experiments showed that the patients’ lymphocytes suppressed hematopoiesis. These cells produced a soluble, inhibitory factor that was eventually identified as interferon-γ. Activation of a TH1-type T-cell response has been inferred from immunophenotypic characterization of T cells and excessive production of interferon, tumor necrosis factor, and interleukin-2. Detection of intracellular interferon-γ in patient samples by flow cytometry may correlate with responsiveness to immunosuppressive therapy and may predict relapse (8). Altered immunity results in destruction, specifically Fas-mediated CD34 cell death, and in activation of intracellular pathways leading to cell-cycle arrest. Immunity is local and has been modeled in tissue culture when low concentrations of interferon-γ are secreted into the marrow microenvironment. In an animal model, bone marrow failure after injection of alloreactive lymphocytes can be prevented by treatment with a monoclonal antibody to interferon-γ (9).

The nature of the antigen or antigens driving the pathologic immune response is unknown. At the molecular level, lymphocytes in aplastic anemia show similarity to T cells in multiple sclerosis, diabetes, and other related illnesses. Characterization of the T-cell–receptor β chain of activated lymphocytes shows that the immune response is oligoclonal, with a relatively limited number of active clones infiltrating the marrow. Some immune responses may be public, that is, shared among patients with the same histocompatibility background (10). Immortalized T-cell clones in the laboratory should allow the definition of antigenic peptides, and knowledge of the antigen-binding sequence of the involved T-cell–receptor β chain could provide a molecular method to distinguish patient subgroups and to assess the effectiveness of immunosuppressive therapies.

The Difficult Differential Diagnosis of Bone Marrow Failure

The presence of fatty bone marrow on biopsy indicates aplasia; however, marrow hypocellularity can occur in other hematologic diseases. New diagnostic tests have profoundly affected the differential diagnosis and our understanding of bone marrow failure (Figure 1). The distinction between
Figure 2. Pathophysiology and treatment of aplastic anemia.

Hypocellular Myelodysplasia

Cytogenetic testing of marrow cells is routine, but the interpretation of results can be controversial. Chromosomes are normal in typical aplastic anemia, but aneuploidy or structural abnormalities are relatively common in the myelodysplastic syndromes. When the marrow is normal or hypercellular and hematopoietic cells are obviously dysmorphic, myelodysplasia is easily distinguished from aplastic anemia. However, in perhaps 20% of cases, marrow samples are hypocellular; furthermore, morphologic changes may be subtle or unconvincing, and chromosome testing may have normal results or be unsuccessful because of low numbers of cells (12). The differential diagnosis is further confused by the evolution of treated aplastic anemia to myelodysplasia (see following discussion) and the responsiveness of patients with some forms of myelodysplasia (especially when bone marrow failure rather than preleukemia is the dominant clinical feature) to the same types of immu-
Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria

A very strong clinical relationship exists between aplastic anemia and paroxysmal nocturnal hemoglobinuria (PNH). In PNH, an abnormal hematopoietic stem cell gives rise to an expanded population of mature red blood cells, granulocytes, and platelets, all of which lack an entire class of distinctive cell-surface proteins. These proteins attach to the cell not through the usual transmembrane hydrophobic domain but by a glycolipid moiety that is covalently bound to the protein after it is synthesized. The genetic basis of PNH is an acquired mutation in PIG-A, an X-chromosome gene that aborts synthesis of the glycosylphosphatidylinositol (GPI) anchor structure. Deficiency of the GPI-anchored protein CD59 explains intravascular hemolysis in PNH, which results from the inability of erythrocytes to inactivate the complement. Absence of GPI-anchored proteins is easily detected by flow cytometric methods applied to both erythrocytes and leukocytes; the Ham and sucrose tests are now obsolete.

It has long been known that some patients with PNH will develop bone marrow failure and, conversely, that PNH may be observed as a “late clonal event” years after the diagnosis of aplastic anemia (see following discussion). However, flow cytometry has revealed that a large proportion of patients with bone marrow failure have expansion of a hematopoietic PNH clone at clinical presentation. In my group’s most recent analysis, PNH cells were identified in 42% of patients with aplastic anemia (and 23% of those with myelodysplasia) early in the disease process and before any treatment (14). Indeed, PNH is only occasionally a late occurrence in aplastic anemia. Conversely, even patients with predominantly hemolytic PNH have evidence of hematopoietic insufficiency in progenitor cell assays. Given this striking degree of clinical and functional overlap, it hardly seems accurate to continue to describe PNH as only an association with aplastic anemia.

What accounts for the expansion of the progeny of a genetically altered stem-cell population in an immune-mediated disease? The results of “knock-out” of the homologous pig-a gene in the mouse, as well as the finding of tiny PNH clones in many normal persons, indicate that the somatic mutation is required but not sufficient to explain clonal expansion. That clonal expansion may be linked to the immune pathophysiology is implied by the strong association of HLA-DR2 with PNH and by the presence of a PNH clone as a positive predictive factor for responsiveness to immunosuppressive therapy (15). However, the exact relationship is unclear. One attractive explanation is resistance of the PNH clone to immune attack, which would produce a selective advantage for these cells. This hypothesis is supported by the finding that in a patient with PNH, the PNH cells appear to be capable of normal growth, but the normal bone marrow cells are at a proliferative disadvantage and appear to be dying as a result of Fas-mediated apoptosis (16, 17). A second possibility is that the GPI-anchored proteins play an antigenic role in the initiation and maintenance of the immune attack on hematopoietic cells (18). An individual protein may be a common culprit, but GPI-anchored proteins may be more globally immunogenic, perhaps resulting from altered degradation of proteins that are retained in the cytoplasm in PIG-A–deficient cells rather than being displayed on the cell surface as in normal cells, with presentation of their peptides in a class I rather than class II histocompatibility antigen context.

AN INFECTIOUS CAUSE FOR APLASTIC ANEMIA?

Mainly on the basis of epidemiologic data, diseases such as multiple sclerosis and type 1 diabetes mellitus have been suspected of having infectious triggers. Animal models and in vitro data support various mechanisms that might lead to a breakdown in tolerance for normal tissues after infection, including molecular mimicry, antigenic spread, and danger signals elicited by a
microbe. For immune-mediated bone marrow failure, one clinical syndrome strongly suggests an inciting infectious cause. Post-hepatitis aplastic anemia typically occurs in young, previously healthy males who have self-limited but severe liver inflammation with very high serum aminotransferase and bilirubin levels; profound pancytopenia follows several weeks later (19). The blood cell count depression is so severe and sustained that post-hepatitis aplastic anemia has been considered an absolute indication for early stem-cell transplantation; however, both the marrow and liver improve with immunosuppressive therapies as well.

The presumed infectious cause of aplastic anemia remains mysterious. Serologic testing consistently yields negative results for the known hepatitis viruses (A, B, and C) and for putative agents, such as hepatitis G and transfusion-transmitted viruses (20). Parvovirus B19 has been implicated in a few cases. This virus directly affects blood cell production but almost always only as a result of highly specific infection of erythroid progenitor cells (21). B19 parvovirus can also occasionally produce mild hepatitis, but its role in more fulminant hepatitis remains uncertain (22).

Seronegative fulminant hepatitis is the most common cause of liver failure in children, and, remarkably, more than one third of patients undergoing transplantation for this form of liver failure develop peri-transplantation aplastic anemia or marrow depression (23). Acute seronegative hepatitis also is often associated with serologic evidence of immune activation, particularly autoantibodies (24). Acute seronegative hepatitis is infrequent in the United States but is more common in liver clinics in developing countries; this is consistent with a presumed enteric mode of transmission. In Hanoi, for example, acute seronegative hepatitis accounted for about 20% of cases (25) compared with less than 3% in the United States (26). This geographic variation parallels rates of aplastic anemia. Large prospective studies established an annual incidence of 2 new cases per 1 million population in Europe and Israel. In Asia, recent similarly conducted studies in Thailand (27) and China (28) have determined the incidence to be about three times that in the West.

**CHOOSING TREATMENTS**

The underlying pathology of acquired aplastic anemia has been addressed by replacing the marrow through stem-cell transplantation or by quelling lymphocyte attack through immunosuppressive therapies (Figure 2). These approaches differ markedly in applicability, short- and long-term risks (29, 30), and the completeness and durability of hematologic recovery.

**Hematopoietic Stem-Cell Transplantation**

Bone marrow and, more recently, peripheral blood stem-cell transplantation from a histocompatible sibling usually cure the underlying bone marrow failure (Figure 3). Survival rates have been reported to be as high as 90% from a single, experienced institution (33) and at 75% to 80% for registry data, which reflect more general experiences (34). Mortality rates for the first 100 days after transplantation have decreased, probably as a result of less graft rejection and better control of infections. Graft-versus-host disease, the frequency and severity of which correlate with patient age, continues to limit the success of transplantation. In most analyses, adults have lower survival rates compared with children. In one large study, 41% of 212 patients surviving for more than 2 years after transplantation had developed chronic GVHD, and the mortality rate was three times higher than the rate for patients without this complication (34); GVHD contributed to earlier deaths as well.

Allogeneic transplantation is available to only a minority of patients because about 70% will lack a suitably matched sibling donor. Many more donors are available outside the family and can now be located through large registries in the United States and Europe. Relatively good results have been achieved at Children’s Hospital in Milwaukee, Wisconsin, where treatment consists of T-cell depletion of the graft combined with cytosine arabinoside, cyclophosphamide, and total-body irradiation. Survival at a median follow-up of about 3 years in 28 children was 54%, despite the heavy transfusion burden and previous treatment; GVHD occurred infrequently (35, 36). Results elsewhere have been more disappointing, especially in adults, as a result of high rates of graft rejection, GVHD, and infection caused by delayed immune system reconstitution. In general, survival has been about half that observed with standard transplants: 29% (37) to 37% (38). Older patients poorly tolerate the rigorous conditioning regimens required for engraftment. Even in children, these regimens are likely to exact a delayed toll of late malignant disease. In aplas-
Figure 3. Results of treatment in patients with acquired aplastic anemia.

A. Allogeneic bone marrow transplantation. Data are presented from individual hospital series in peer-reviewed publications from 1991 to 1997. The shaded area represents the 5-year probability of survival (with the same confidence intervals) of patients reported to the International Bone Marrow Transplant Registry (IBMTR) during this period. Adapted with permission from Horowitz (31); original source provides detailed information on each series.

B. The continuing influence of age on survival, as reflected in IBMTR data. Adapted with permission from Horowitz (31).

C. Comparative probability of survival after immunosuppression and bone marrow transplantation. The data are for patients reported to the Working Party on Severe Aplastic Anemia of The European Group for Blood and Marrow Transplantation in the 1980s and 1990s. Adapted with permission from Bacigalupo et al. (32); CSA = cyclosporine; FHCRC = Fred Hutchinson Cancer Research Center; MTX = methotrexate; UCLA = University of California, Los Angeles.
tic anemia, malignant tumors occur at a higher-than-expected rate in patients undergoing standard conditioning (39, 40); intensive chemotherapy and radiation therapy used in unrelated-donor regimens may increase this risk (41).

Immunosuppression

Immunosuppression is used in patients who are not candidates for stem-cell transplantation because of older age or lack of a donor. Both horse and rabbit antithymocyte globulin (ATG) are licensed in the United States. Hematologic responses, which are usually equivalent to sufficiently improved blood cell counts, occur in 40% to 50% of patients treated with ATG; such patients no longer require transfusions and are not susceptible to infection (29, 42). The addition of cyclosporine to ATG has improved response and survival. In studies in Europe (43) and the United States (44), response rates have been 70% to 80%, and the 5-year survival rate in the responding patients has been 80% to 90%. Combined treatment with cyclosporine and ATG versus ATG alone shows substantial benefit for children and patients with absolute neutropenia. As a single agent, cyclosporine is inferior to ATG (45).

Many patients with aplastic anemia are not adequately treated with a single course of ATG followed by several months of cyclosporine. Relapse is not infrequent. Blood cell counts may decrease when cyclosporine is discontinued but then increase when the agent is reinstituted. In some patients, maintaining blood cell counts may depend on continued administration of low-dose cyclosporine. Frank recurrence of pancytopenia prompts a second course of ATG. However, long-term prognosis does not appear to be affected by relapse. Patients who respond to immunosuppression often continue to have blood cell counts that, while adequate for full activities, remain below normal. Incomplete responses, frequent relapses, and cyclosporine dependence are most likely evidence of chronic immune system effects on a hematologically compensated bone marrow. An additional problem is the development of late clonal diseases (see following discussion).

The effectiveness of immunosuppressive therapy in aplastic anemia was first observed when ATG was used in the conditioning regimen before transplantation: Some patients who rejected the graft recovered their own marrow function. The same effect was seen with cyclophosphamide. Small numbers of patients at Johns Hopkins University, Baltimore, Maryland, were treated with high-dose cyclophosphamide without stem-cell rescue during intervals in the 1980s, when ATG was temporarily not available; results were promising (46). Outcomes of the same treatment in 19 patients have recently been published (47). The hematologic response rate was similar to that seen with ATG combined with cyclosporine; more important, neither relapse nor evolution to PNH or myelodysplasia was observed. However, a randomized trial conducted at the National Institutes of Health (NIH) that compared ATG with cyclophosphamide (both study groups included cyclosporine) was terminated prematurely because of excessive toxicity, severe fungal infections, and deaths in the group that received cyclophosphamide (48). Cyclophosphamide is a much more aggressive immunosuppressive treatment than ATG. Absolute neutropenia can persist for many weeks, necessitating prolonged courses of antibiotics and even granulocyte transfusions. Thus, extremely severe disease, with its poorer immediate prognosis, may be iatrogenically induced. It is disappointing that some patients in the NIH study who received high-dose cyclophosphamide have relapsed or developed cytogenetic abnormalities and that PNH clones were unaltered by treatment (49).

One new strategy is based on inducing immunologic tolerance (Figure 4). Antithymocyte globulin reduces lymphocyte counts, but transiently and modestly compared with the effect of cytotoxic chemotherapy. Part of the beneficial activity may be the induction of tolerance, perhaps by specific deletion of activated lymphocytes. Indeed, the concurrent use of cyclosporine, which blocks T-cell activation, may blunt the efficacy of ATG (50, 51). In our new protocol, we delay the introduction of cyclosporine and add a novel immunosuppressive drug, mycophenolate mofetil. This drug, inhibiting inosine monophosphate, is cytotoxic for cycling T cells. Activated lymphocytes should be subject to elimination by their characteristic cell-surface antigens (recognized by ATG) and their mitotic activity.

Other mild but more specific forms of immunosuppression might also be effective. For example, ATG contains antibody specificities for the interleukin-2 receptor, which is present on activated lymphocytes. My colleagues and I are testing a commercially available monoclonal anti-
body to this receptor in patients with moderate aplastic anemia. Monoclonal antibodies, recombinant soluble cytokine receptors, and new immunosuppressive drugs (for example, rapamycin) deserve examination in immune-mediated bone marrow failure syndromes.

Refractory Pancytopenia

One quarter to one third of patients do not respond to ATG and cyclosporine. In addition, in my most recent analysis of 122 patients receiving this protocol, both survival and evolution to late clonal diseases in patients who responded were inversely correlated with the degree of improvement in blood cell counts at 3 months (52). At present, no clear guidelines are available for the treatment of refractory or poorly responsive aplastic anemia. Multiple courses of immunosuppression are commonly administered at European centers. In a recent study from Italy, most patients who received rabbit ATG after unsuccessful treatment with horse ATG became transfusion independent (53). These results suggest that primary treatment failure should not necessarily be interpreted as indicating a nonimmune mechanism of disease. Nevertheless, in some patients, the marrow may be too severely damaged to allow recovery. Because of the poor long-term outlook, patients in whom immunosuppression fails should consider unrelated stem-cell transplantation.

There is little justification for either a therapeutic trial of corticosteroids as primary treatment or for their long-term use to prevent bleeding. Patients with aplastic anemia seem to be particularly susceptible to one of the severe complications associated with corticosteroid use—aseptic necrosis (54). Hematopoietic growth factors are not appropriate as first-line treatment for severe aplastic anemia. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor can increase granulocyte counts in aplastic anemia, but this effect is almost always transient and rarely occurs in patients with profound neutropenia (55). In addition, G-CSF does not improve the response or survival rates in patients receiving standard immunosuppressive therapy (56). However, growth factors can occasionally be useful in refractory aplastic anemia. Clinically meaningful improvement in blood cell counts occasionally has been seen after prolonged administration of G-CSF, erythropoietin, and stem-cell factor, usually in some combination (57–59).

A few patients have a choice of allogeneic transplantation or immunosuppressive therapy. For patients who are “biologically” assigned to transplantation because
they have an appropriately matched sibling, morbidity and mortality rates have been compared with those of patients receiving immunosuppression (although this is an imperfect surrogate for a true randomized, controlled trial because medical and socioeconomic factors may preclude a specific treatment). Analyses of large databases have not shown major general differences in outcomes between the two therapeutic approaches (Figure 3C) (32). Nevertheless, transplantation is preferable for certain defined subgroups: most children and patients with very severe neutropenia. Patients in whom immunosuppressive therapy fails have later undergone successful transplantation from matched siblings or from unrelated donors.

**EVOLUTION OF APLASTIC ANEMIA TO OTHER HEMATOLOGIC DISEASES AND LATE CLONAL EVENTS**

As patient survival has improved, so too has the opportunity to observe the long-term course of bone marrow failure. Evolution of aplastic anemia to another hematologic disease has been reported in a substantial minority of patients undergoing immunosuppressive therapy. In a large European series of more than 200 patients, the actuarial risk for developing myelodysplasia and leukemia at 7 years was 15% (60). In a series of more than 100 patients closely followed at the NIH Clinical Center, my colleagues and I found the actuarial risk for late clonal events among responders at 6 to 10 years after treatment to be 19%. It should be noted that immunosuppression by itself is not the cause of late clonal events; similar clinical progression has been seen in patients treated with androgens (61).

Appearance of a dysmorphic, cellular marrow on an aspirate smear with or without new chromosomal abnormalities leads to the diagnosis of myelodysplasia. The original diagnosis of aplastic anemia may be questioned when such an abnormal bone marrow is observed within weeks or a few months after presentation or treatment. In addition, aplastic anemia and myelodysplasia may share the same immune pathophysiology for the development of pancytopenia. Young patients with some forms of myelodysplastic syndromes, especially those with hypocellular marrow and normal cytogenetic findings, respond well to ATG or cyclosporine (62). As in aplastic anemia, laboratory data can show lymphocyte activation and excessive production of lymphokines, with resulting increased apoptosis of hematopoietic cells (13).

The significance of specific cytogenetic findings in aplastic anemia varies. Some chromosomal abnormalities may be only transient. Patients with trisomy 8 are often dependent on a long-term course of cyclosporine to maintain normal blood cell counts. Their prognosis is relatively good. Monosomy 7, in contrast, is almost always a harbinger of refractory pancytopenia and carries a high likelihood of leukemic transformation.

Chromosomal abnormalities can develop years after blood cell counts have improved with immunosuppressive therapy, even though results of intervening multiple cytogenetic studies were normal. This pattern suggests genomic instability. A relationship between chronic inflammation and malignancy has been a subject of speculation and study for many decades. Analogous clinical relationships exist between chronic hepatitis and hepatocellular carcinoma, ulcerative colitis and colon cancer, and chronic GVHD and various malignant tumors. In aplastic anemia, abnormal clones may be selected for expansion under conditions of hematopoietic stress (for example, sustained high hematopoietic growth-factor concentrations). Alternatively, a chronic inflammatory environment may itself be mutagenic as a result of increased concentrations of reactive oxygen species or cell damage short of complete apoptosis.

**ACQUIRED APLASTIC ANEMIA AS A HEMATOLOGIC DISEASE AND AS AN IMMUNE-MEDIATED SYNDROME**

In the 20th century, our understanding of the origin of aplastic anemia and the definitive and supportive treatment of patients improved enormously. Unfortunately, stem-cell destruction may be very advanced by the time the patient presents with pancytopenia. Restoration of hematopoiesis by stem-cell transplantation or suppression of the pathologic immune response to allow the patient’s own marrow to recover are both effective. Improvements in methods for monitoring hematopoiesis and immune system activity and especially in the use of immunomodulatory drugs should be of clinical benefit. Major research questions remain concerning the nature of inciting antigens and the determinant of the aberrant immune response, as well as the fundamental pathophysiologic relationships among aplasia, dysplasia, and PNH.
Acquired aplastic anemia is usually immune mediated. It shares clinical and epidemiologic features and pathophysiologic mechanisms with other human autoimmune diseases in which restricted numbers of T-cell clones effect severe, tissue-specific destruction. Autoimmune diseases occur in young persons, often with only a modest familial disposition, if any. Geographic variation may be observed, and precipitating events (for example, viral infection) have been suggested because of the peculiar epidemiologic features of autoimmune diseases. Studies in humans and animal models have suggested that limited numbers of antigens may drive this highly efficient and selective destructive process. The affected target cells have determined not only the clinical presentation but also the prognosis for recovery or irreparable loss of organ function and relapse. Historically, emphasis on end-organ damage has meant that different subspecialists have confronted each of these diseases. Improvement in our understanding and especially in our ability to measure the underlying pathophysiologic mechanisms—combined with the development of new immunosuppressive and immunomodulatory drugs—suggests that the common immunologic pathways of organ destruction can be addressed across subspecialty boundaries.

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