

Review Article

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor*THE PATHOPHYSIOLOGY OF
ACQUIRED APLASTIC ANEMIANEAL S. YOUNG, M.D.,
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APLASTIC anemia, which is pancytopenia with a fatty or “empty” bone marrow, is remarkable for the simplicity of its pathologic picture and the direct derivation of its clinical manifestations.¹ Although it is not a common disease, the drama of an individual case and the larger consequences of its associations give it considerable interest. That aplastic anemia is perhaps the most dreaded idiosyncratic complication of drug treatment has serious and often expensive consequences for drug development, for risk assessment, for approval by regulatory agencies, and in legal actions.

Aplastic anemia, first described by Paul Ehrlich in 1888 from an autopsy of a young pregnant woman who had died after a brief catastrophic illness, is the clearest example of failed hematopoiesis in humans. It differs from agranulocytosis and pure red-cell aplasia, which involve only granulocyte and erythrocyte production, respectively; from myelodysplasia, in which marrow morphology is abnormal and chromosomal abnormalities are common; and from Fanconi's anemia, in which aplastic anemia is inherited. In all these disorders, the immune system may influence hematopoiesis. An immunologic basis for agranulocytosis was established by the reproduction of the syndrome by challenging affected patients with drugs or by infusing plasma from patients into normal subjects. Patients with pure red-cell aplasia have antibodies against erythroid precursor cells and lymphocytes capable of inhibiting erythropoiesis. Some patients with myelodysplasia respond to immunosuppressive therapy, and in Fanconi's anemia, sus-

ceptibility to an immune cytokine may be a marker of the genetic defect.

We argue here that in most patients with acquired aplastic anemia, bone marrow failure results from immunologically mediated, tissue-specific organ destruction. The course of the disease can be separated into distinct phases (Fig. 1). After exposure to an inciting antigen, cells and cytokines of the immune system act destructively on stem cells in the marrow, reducing their number so that normal levels of circulating leukocytes, erythrocytes, and platelets are not maintained. Immunosuppressive therapies can reverse this process, leading to improved marrow function and partial or full resolution of pancytopenia, often without an increase in the number of stem cells. Late complications of aplastic anemia include not only relapse of pancytopenia but also evolution of other clonal and sometimes malignant hematologic conditions from the injured hematopoietic-cell compartment.

HEMATOPOIESIS
IN BONE MARROW FAILURE

By any measure, hematopoiesis is greatly impaired in aplastic anemia. By definition, there is pancytopenia, and the most severely affected patients have neutrophil counts of less than 200 per cubic millimeter, platelet counts of less than 20,000 per cubic millimeter, and reticulocyte counts of less than 60,000 per cubic millimeter. The precursors of these circulating cells normally comprise most of the cells seen in a histologic preparation of bone marrow. However, in aplastic anemia, there are very few early erythroid and myeloid cells at any stage of differentiation, and megakaryocytes are scanty if present at all. Primitive progenitor and stem cells, which normally constitute about 1 percent of marrow cells, cannot be identified by their appearance. These cells express a cytoadhesive protein called CD34; according to fluorescent flow-cytometric assay, the number of CD34 cells is much reduced in aplastic anemia.^{2,3} Hematopoietic progenitors of mature red and white cells and megakaryocytes, which can be quantitated in functional tests of colony formation, are virtually absent.^{2,3}

Stem Cells

Hematopoietic stem cells are characterized by high proliferative capacity, the potential to differentiate along all lineage pathways, and the property of self-renewal (the ability to generate additional stem cells by mitosis without differentiation). Stem cells are rigorously defined by their ability to repopulate the hematopoietic compartment of lethally irradiated

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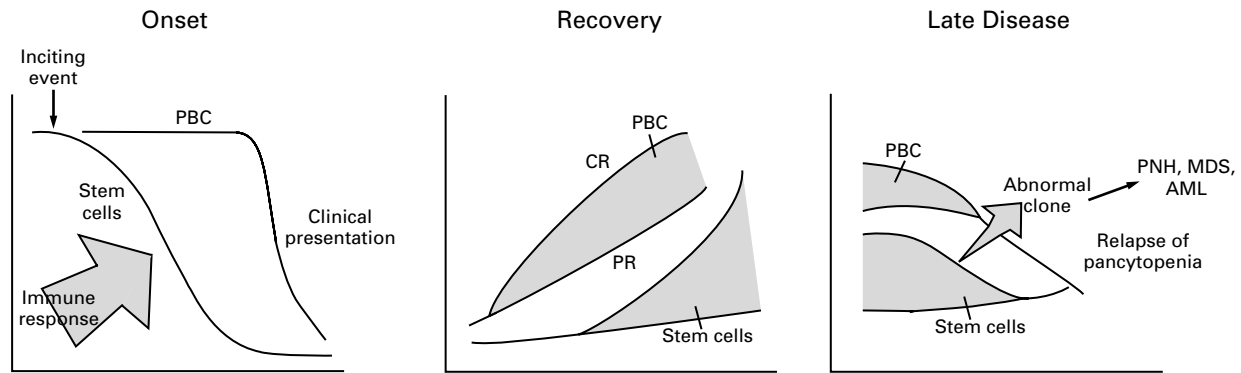


Figure 1. Stem-Cell Counts and Peripheral-Blood Counts (PBC) during the Course of Immune-Mediated Aplastic Anemia.

The left-hand panel shows the onset of the disease. After an inciting event, such as drug exposure or viral infection, the hematopoietic compartment is destroyed by the immune system. Small numbers of surviving stem cells support adequate hematopoiesis for some time, but eventually the cell counts become very low and symptoms appear. The middle panel shows the recovery of blood counts. Either a partial response (PR) or a complete response (CR) can occur, at least initially, without increased numbers of stem cells. In some patients, the primitive-cell compartment appears to repopulate over time by the process of self-renewal of stem cells. The right-hand panel shows late disease. Years after recovery, blood counts may fall as a relapse of pancytopenia occurs, or an abnormal clone of stem cells may emerge, leading to a new diagnosis of paroxysmal nocturnal hemoglobinuria (PNH), myelodysplasia (MDS), or acute myelogenous leukemia (AML).

animals. In humans, the basis of an *in vitro* stem-cell assay is colony formation after prolonged bone marrow culture; these long-term culture-initiating cells have the frequency, phenotype, and kinetic properties of true stem cells. Recent studies indicate a profound deficit in these primitive progenitors in all patients with severe aplastic anemia.^{4,5} At the time of clinical presentation, the number of these cells in marrow samples is usually less than 10 percent of normal, and the absolute number of stem cells is probably no more than 1 percent of normal.

Stroma and Hematopoietic Growth Factors

The survival and proliferation of hematopoietic cells are dependent on stromal cells, which provide growth factors that are essential for the viability, proliferation, and differentiation of stem cells and progenitor cells. Successful bone marrow transplantation in patients with aplastic anemia implies adequate stromal function, because important stromal elements of host origin remain. In the laboratory, stromal cells from patients with aplastic anemia usually are not defective. For example, they support hematopoiesis by normal CD34 cells, whereas no hematopoietic colonies develop when the patients' CD34 cells are cultured on normal stroma.^{6,7} Moreover, stromal cells from patients with aplastic anemia produce normal or increased quantities of hematopoietic growth factors *in vitro*.⁸⁻¹⁰ The failure of macrophages from patients with aplastic anemia to make interleukin-1 *in vitro* may reflect a more general maturation defect of monocytes. Marrow stroma from a minority of patients produces only small amounts of granulocyte-macrophage colony-stimu-

lating factor,¹¹ interleukin-3, and granulocyte colony-stimulating factor,¹² but serum concentrations of erythropoietin,¹³ thrombopoietin,¹⁴ and granulocyte colony-stimulating factor¹⁵ are usually greatly elevated.¹⁶ Cytokines that act at very early stages of hematopoiesis include Flt-3 ligand, serum concentrations of which are elevated in aplastic anemia,¹⁷ and stem-cell factor, concentrations of which are only slightly decreased.¹⁸

Treating aplastic anemia with hematopoietic growth factors — even with those that are putatively deficient in the disease — has not been very effective in restoring hematopoiesis.¹⁹ In most trials, administration of granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and interleukin-3 resulted in only small and transient increases in the number of granulocytes; interleukin-1 has been ineffective. Thus, the results of many clinical and laboratory studies argue against growth-factor deficiencies as a cause of most cases of aplastic anemia.

MEDICAL THERAPY AND INFERENCES ABOUT THE MECHANISM OF DISEASE

Direct Hematopoietic Injury

The commonest form of aplastic anemia is iatrogenic — the transient marrow failure that follows cytotoxic chemotherapy or radiotherapy (Table 1). Certain chemical or physical agents directly injure both proliferating and quiescent hematopoietic cells, leading to damage to DNA and ultimately to apoptosis. However, patients with community-acquired aplastic anemia rarely have a history of exposure to

any substance that is toxic to the bone marrow, and even benzene is now infrequently associated with aplastic anemia in developed countries.²⁰

In general, drug toxicity is mediated through intermediate metabolites that bind covalently to protein and DNA. These reactive metabolites are both formed and degraded by complex metabolic pathways, and genetic variation in the responsible enzymes could contribute to the rarity of idiosyncratic drug reactions.¹ Benzene has a more consistent effect on the marrow in humans and animals. In rodents, benzene can be metabolized by marrow cells,^{1,21,22} and both benzene and its metabolites bind covalently to marrow-cell proteins and DNA.²³ The myeloperoxidase in granulocytes and myeloid progenitor cells generates toxic quinone metabolites from benzene, and bone marrow has lower levels of quinone-detoxifying reductase than liver.²¹

Immune-Mediated Bone Marrow Failure

The unexpected improvement of pancytopenia in patients after failed allogeneic bone marrow transplantation led to the suggestion that the immunosuppressive conditioning regimen that was intended to allow engraftment of the donor marrow might instead have promoted the function of the host marrow.²⁴ The return of blood-cell production by the patient's own marrow occurred after the administration of antilymphocyte globulin or cyclophosphamide. Moreover, to succeed in restoring hematopoiesis, marrow transplantation from an identical twin usually requires immunosuppressive preparation, despite the genetic identity of the donor and recipient.²⁵

These observations prompted trials of immunosuppressive therapy for aplastic anemia, first with antilymphocyte globulin in Europe,²⁶ and then with antithymocyte globulin in the United States,²⁷ with high doses of methylprednisolone,²⁸ cyclosporine,²⁹ or cyclophosphamide.³⁰ The most successful regimens have combined antilymphocyte globulin and cyclosporine. In recent large studies, the rates of success, defined as no further need of transfusion and neutrophil numbers adequate to prevent infection, were 70 to 80 percent.^{31,32} Long-term survival after these treatments has equaled that after allogeneic bone marrow transplantation.¹⁹ The fact that these medical treatments reduce the numbers of lymphocytes or block T-cell function, together with the superior results with combinations of these drugs, strongly suggests that immunosuppression accounts for their success.

IMMUNE DESTRUCTION OF HEMATOPOIETIC CELLS

A model for the interaction between the immune system and hematopoietic cells in patients with aplastic anemia has been developed from laboratory observations (Fig. 2). An early experiment showed

TABLE 1. AN ETIOLOGIC CLASSIFICATION OF APLASTIC ANEMIA.*

Direct toxicity
Iatrogenic causes
Radiation
Chemotherapy
Benzene
Intermediate metabolites of some common drugs
Immune-mediated causes
Iatrogenic causes
Transfusion-associated graft-versus-host disease
Eosinophilic fasciitis
Hepatitis-associated disease
Pregnancy
Intermediate metabolites of some common drugs
Idiopathic aplastic anemia

*Boldface type indicates relatively well established mechanisms.

that mononuclear cells from the blood or marrow of patients with aplastic anemia suppressed hematopoietic colony formation by normal marrow cells; the removal of T cells from patients' samples sometimes improved in vitro colony formation by the affected bone marrow.³⁵ One inhibitory activity in the supernatants of cultures of patients' cells proved to be interferon- γ . Patients' T cells in bulk culture³⁶ or when cloned³⁷ overproduced both interferon- γ and tumor necrosis factor, two cytokines that inhibit hematopoietic colony formation in vitro.³⁸ Interferon- γ messenger RNA was detectable in samples of marrow from most patients.^{39,40} **Blood and marrow from patients also contained increased numbers of activated cytotoxic lymphocytes,**⁴¹ and the activity and numbers of these cells decreased with successful antithymocyte globulin therapy.^{42,43}

In tissue culture, interferon- γ and tumor necrosis factor suppress the proliferation of early and late hematopoietic progenitor cells and stem cells.³⁸ This suppression is greater when the interferon- γ and tumor necrosis factor are secreted into the marrow microenvironment than when they are added to cultured cells.⁴⁴ Interferon- γ and tumor necrosis factor suppress hematopoiesis by effects on the mitotic cycle, but an important component of inhibition is cell killing. Both induce expression of the Fas receptor on CD34 cells; activation of this receptor by its ligand initiates apoptosis.^{45,46} Hematopoietic cells of patients with aplastic anemia express Fas receptors,⁴⁷ and the marrow contains increased numbers of apoptotic cells.⁴⁸ Activation of a number of functionally important genes by signal transduction through the pathways triggered by binding of Fas ligand, interferon- γ , and tumor necrosis factor influences both the cycling and viability of hematopoietic cells (Fig. 2).

The immunologic events that precede the destruction of hematopoietic cells are much less clear.

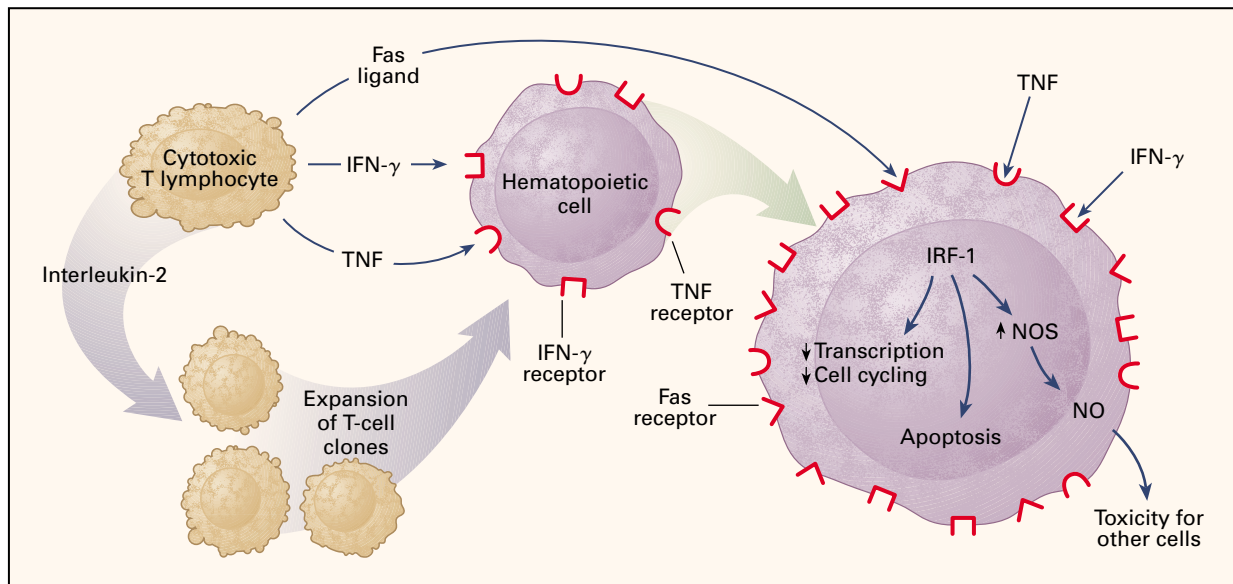


Figure 2. Immune Destruction of Hematopoiesis.

Hematopoietic-cell targets are affected by ligand–receptor interactions, intracellular signaling, and gene activation. Activated cytotoxic T lymphocytes have a major role in tissue damage, acting at least in part through secreted lymphokines like interferon- γ (IFN- γ) and tumor necrosis factor (TNF). IFN- γ and TNF can up-regulate each other's cellular receptors and also the Fas receptor. Increased production of interleukin-2 leads to polyclonal expansion of T cells. Activation of the Fas receptor by the Fas ligand present on lymphocytes leads to apoptosis in target cells. Some effects of IFN- γ are mediated through interferon regulatory factor 1 (IRF-1),³³ 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.³⁴ Direct cell–cell interactions between effector lymphocytes and targets probably also occur.

Involvement of lymphocytes of the CD4 or helper class has been inferred from overrepresentation of the class II histocompatibility antigen HLA-DR2 in white patients with aplastic anemia,⁴⁹ and a more specific HLA haplotype has been linked to the disorder in Japanese patients.⁵⁰ Some HLA antigens may be much more common in subgroups of patients with aplastic anemia — for example, in those who respond to cyclosporine⁵⁰ or those who have bone marrow failure after hepatitis.⁵¹ Clones of HLA-DR–restricted T cells have been shown to proliferate in response to autologous marrow cells.^{52,53} However, both the dysregulatory events that lead to loss of tolerance and to autoimmune destruction of hematopoietic cells and the initial antigen exposure that triggers immune system activation are unknown.

INCITING EVENTS

Immune destruction of marrow occurs in animals with acute graft-versus-host disease and in humans with transfusion-associated graft-versus-host disease, in whom marrow destruction is invariably the cause of death.⁵⁴ Under these special conditions, graft-versus-host disease can be initiated by very small numbers of effector cells, such as the lympho-

cytes present in plasma or solid-organ transplants, and only a single amino acid difference in HLA molecules is sufficient to induce graft-versus-host disease.

Hepatitis, Aplastic Anemia, and Viruses

Severe pancytopenia can occasionally occur one to two months after an episode of apparent viral hepatitis. The stereotypical syndrome of post-hepatitis aplasia would seem to offer the opportunity to identify a specific infectious cause of aplastic anemia. In most patients, the hepatitis is non-A, non-B, non-C, and non-G.⁵¹ Although aplastic anemia is a rare sequela of hepatitis, there is a striking relation between fulminant seronegative hepatitis and aplastic anemia. In about one third of patients who undergo liver transplantation for this cause of liver failure, marrow failure occurs in the peritransplantation period.⁵⁵ There is marked activation of cytotoxic lymphocytes in patients with post-hepatitis aplastic anemia, and they respond favorably to immunosuppressive drug therapy, with rapid resolution of abnormal liver function and slower recovery of marrow function.⁵¹ These observations provide evidence of an immune pathophysiology (Fig. 3).

The results of epidemiologic studies suggest the in-

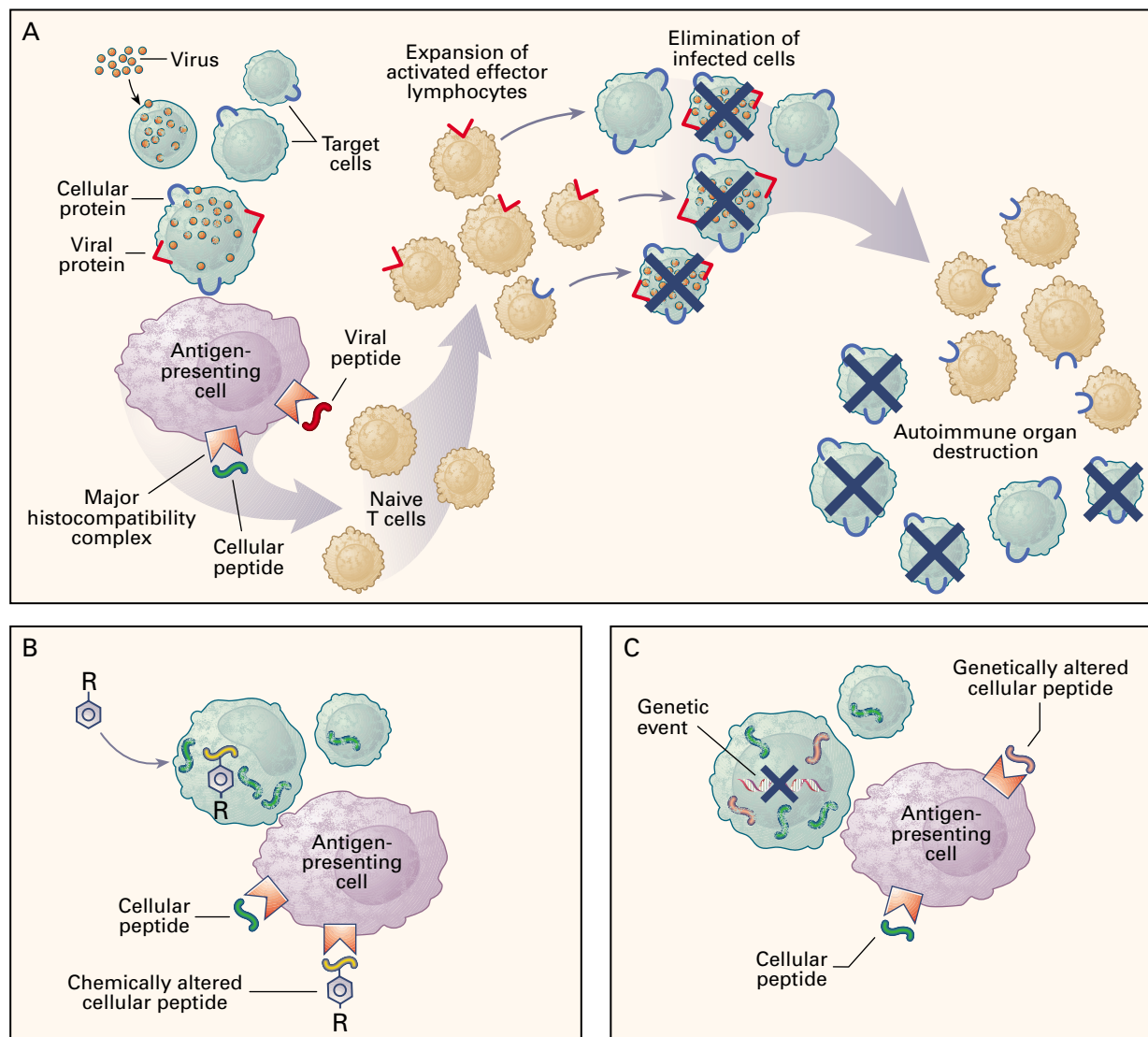


Figure 3. Antigenic Stimuli Initiating the Destruction of Bone Marrow.

A virus (Panel A) may infect hematopoietic cells (or hepatocytes in hepatitis), leading to the production of novel viral proteins and the excessive or aberrant production of normal cellular proteins, which are released and then taken up by antigen-presenting cells, in which the proteins are processed to become peptides, form complexes with major-histocompatibility-complex molecules, and are presented to naive T cells. The immune response results in the destruction of infected cells. In rare cases, the lymphocyte response persists and affects normal cells, resulting in autoimmune organ destruction. A similar process may occur in patients with drug-induced aplastic anemia (Panel B). Reactive drug metabolites, formed in marrow cells, may bind to cellular proteins, leading the immune system to recognize them as foreign, thereby initiating the sequence shown in Panel A. A generic substituted hydrocarbon is symbolized by a benzene ring with an R moiety. Presentation of an altered or aberrantly expressed protein — for example, a product of a chromosomal translocation or an oncogene (Panel C) — may similarly initiate a T-cell response that broadens to include nonmalignant cells, leading to marrow hypocellularity in myelodysplasia through a similar sequence.

involvement of an enteric microbial agent in the causation of aplastic anemia. Aplastic anemia is not only more common in the Far East, where hepatitis viruses are prevalent, but is also associated with poverty,⁵⁶ rice farming (with attendant exposure to water and insects),⁵⁷ and past (but not recent) exposure to hepatitis A.⁵⁸

Drugs as Antigens

Many drugs have been associated with aplastic anemia, but unlike anticancer agents and benzene, which at sufficient doses regularly result in marrow aplasia, most other drugs cause idiosyncratic reactions, and only rare patients, who may have ingested small amounts of a drug, have the complication of

bone marrow failure. Drug associations with aplastic anemia have been established in prospective, population-based epidemiologic studies. In Europe and Israel in the 1980s, about 25 percent of cases of aplastic anemia were attributed to drugs.²⁰ In Thailand, however, where the incidence of aplastic anemia is higher than in the West,⁵⁹ the proportion of cases attributable to drug use was only 15 percent (and chloramphenicol was not a risk factor).⁵⁸

Although a putative inciting antigen can be implicated on the basis of a history of exposure, drug-induced hematopoietic failure is difficult to study, because it is idiosyncratic. Animal models do not exist. The diversity of implicated drugs and the problem of assigning causation in an individual patient make clinical studies impractical. Antibodies to drugs or cells have only occasionally been identified in patients with aplastic anemia, and links are therefore established by clinical history taking rather than laboratory tests. The clinical characteristics of patients with drug-associated disease, including response to immunosuppressive therapy, are the same as those of patients with idiopathic disease.⁶⁰

In the related syndrome of agranulocytosis, in which only neutrophil production is decreased, an association with drugs is much more common. The strong linkage between particular HLA class II molecules and neutropenia due to clozapine⁶¹ and methimazole⁶² treatment in certain ethnic groups suggests early involvement of CD4 T cells in drug-induced marrow failure. White-cell agglutinating antibodies are present in some patients with agranulocytosis, but not in those with aplastic anemia. Drugs probably do not serve as simple haptens but instead lead to loss of tolerance by binding to cellular proteins (Fig. 3B). The rarity of idiosyncratic drug reactions could be a function of genetic variations in drug metabolism, differences in major histocompatibility antigens and their peptide-binding properties, and the repertoire of circulating lymphocytes during the period of drug exposure.

RELATION TO LATE CLONAL HEMATOLOGIC DISORDERS

An important and as yet unexplained complication in the clinical course of aplastic anemia is the development of late clonal hematologic diseases, often years after successful immunosuppressive therapy. Paroxysmal nocturnal hemoglobinuria occurs in approximately 9 percent of patients,⁶³ and myelodysplasia and acute myelogenous leukemia occur at a cumulative incidence rate of about 16 percent 10 years after treatment.⁶⁴ One hypothesis has postulated that aplastic anemia is primarily preleukemic. Indeed, in some patients, hematopoiesis appears to originate from a limited number of cells,⁶⁵ but oligoclonality may simply reflect the small numbers of stem cells. Genetically abnormal hematopoietic cells

could incite an immune response through the presentation of novel proteins or aberrantly expressed normal proteins (Fig. 3C). Indeed, in a large proportion of patients with myelodysplasia, a disease marked by chromosomal abnormalities in marrow cells, blood counts improve after therapy with antithymocyte globulin.⁶⁶ Nevertheless, there is no evidence of premalignant cells early in the course of aplastic anemia, and the results of cytogenetic studies and more sensitive molecular assays for specific gene mutations are almost always normal initially.⁶⁷

Studies of paroxysmal nocturnal hemoglobinuria suggest an alternative explanation for clonal hematologic disease in aplastic anemia. In this disorder, mutations in a gene termed *PIG-A* result in the failure to present a large class of proteins on the hematopoietic-cell surface.⁶⁸ These proteins share a unique linkage to the surface membrane through a glycolipid structure called the glycosylphosphatidylinositol anchor. Some glycosylphosphatidylinositol-linked proteins inactivate complement on the red-cell surface. This explains the characteristic intravascular hemolysis in the syndrome, but the biochemical basis of marrow failure in patients with paroxysmal nocturnal hemoglobinuria is unknown. In "knockout" mouse models, loss of *PIG-A* gene function conferred no intrinsic advantage to affected hematopoietic cells.^{69,70} Other observations have suggested that clones emerge because they are favored by certain extrinsic conditions. Patients may harbor clones with different *PIG-A* gene mutations, a finding consistent with the independent proliferation of genetically altered hematopoietic stem cells under some selective pressure.⁷¹ Cells with the paroxysmal nocturnal hemoglobinuria phenotype have been detected in patients with lymphoma during treatment with an anti-T-cell monoclonal antibody that coincidentally recognized a glycosylphosphatidylinositol-linked protein, suggesting that clones deficient in this type of protein expression may be normally present in the hematopoietic stem-cell compartment and expand if their proliferation is favored.⁷² Because some of the molecules that serve as cell-surface ligands for lymphocytes are also glycosylphosphatidylinositol-linked, cells that lack these proteins may be able to escape attack by the immune system.

APLASTIC ANEMIA AND OTHER IMMUNE-MEDIATED DISEASES

Most patients with aplastic anemia respond favorably to immunosuppressive therapies, and laboratory studies of patients' lymphocytes and their products support the concept of pathophysiologic roles for lymphocytes and lymphokines in the destruction of hematopoietic cells. Similar pathophysiologic mechanisms have been proposed for multiple sclerosis, insulin-dependent diabetes mellitus, chronic autoim-

mune thyroiditis, uveitis, and idiopathic myocarditis. Like aplastic anemia, these illnesses often occur in younger patients and their prevalence may vary geographically; a viral cause is often suspected. In all these disorders, tissue-specific organ destruction involves activation of cytotoxic T cells, production of cytokines, and death of target cells by apoptosis. For aplastic anemia, a variety of antigens — derived from chemicals, viruses, and perhaps altered self-proteins — have been inferred from clinical histories to initiate the immune process, but the precise nature of the antigenic stimulus has not been identified. Aplastic anemia responds to immunosuppressive therapy, but the limitations to success in treating this disease, like others, appear to be the degree of organ destruction, the capacity for tissue regeneration, and perhaps most important, a pharmacology that is inadequate to control a misdirected and extraordinarily potent immune response.

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